DEVELOPMENT AND EVALUATION OF MICROEMULSION FOR TRANSDERMAL DELIVERY OF LORNOXICAM

Dissertation submitted to

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Chennai - 600 032

In partial fulfillment for the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

By

Reg. No: 26102204

DEPARTMENT OF PHARMACEUTICS

PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES FOR GIRLS

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CERTIFICATE

This is to Certify that the dissertation entitled **"DEVELOPMENT AND EVALUATION OF MICROEMULSION FOR TRANSDERMAL DELIVERY OF LORNOXICAM"** submitted by **Ms. S. NANCYA [Reg No: 26102204]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by her in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli under my guidance and direct supervision.

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This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in **Pharmaceutics**, of The Tamilnadu Dr. M.G.R Medical University, during May 2012.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Expansion
TDDS	Transdermal Drug Delivery System
LX	Lornoxicam
ME	Microemulsion
RA	Rheumatoid arthritis
NSAIDS	Non-Steroidal Anti Inflammatory Drugs
O/W	Oil in Water
W/O	Water in Oil
PBS	Phosphate Buffer Saline
RH	Relative Humidity
TEM	Transmission Electron Microscopy
AFM	Atomic Force Microscopy
UV	Ultraviolet
NMF	Natural Moisturizing Factor
HLB	Hydrophilic Liphophilic Balance
RHLB	Required Hydrophilic Liphophilic Balance
GRAS	Genaral Regarded As Safe
I.P	Indian Pharmacopoeia
USP	United State Pharmacopoeia
FTIR	Fourier Transform Infrared Spectroscopy
IL	InterLukin
COX	Cyclooxygenase
PG	Protaglandin
TNF	Tumour Necrotic Factor
PIT	Phase Inversion Temperature
AUC	Area Under The Curve

mm	milli meter
cm^2	centimeter square
c°	Degree Cecius
cP	centipoises
nm	nanometer
mg	milligram
λ	Lambda
cm	centimeter
g	gram
sec	second
m	meter
μm	micrometer

1. INTRODUCTION

1.1. Transdermal Drug Delivery Systems [1, 2]

Currently, transdermal drug delivery is one of the most promising methods for drug application. Increasing numbers of drugs are being added to the list of therapeutic agents that can be delivered to the systemic circulation *via* skin. Transdermal drug delivery systems (TDDS) can be defined as self contained discrete dosage forms which, when applied to the intact skin, delivers the drug(s) through the skin at a controlled rate to the systemic circulation.

The potential of using intact skin as the route of drug administration has been known for several years. The inspiration of using skin for delivery of drug is from ancient time. Ebers papyrus used the husk of castor oil plant bark imbibed with water placed on aching head. Historically, the medicated plaster can be viewed as the first development of transdermal drug delivery; this medicated plaster became very popular in Japan as over the counter pharmaceutical dosage form

Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous input of drugs with short biological half-life and eliminates pulsed entry into systemic circulation, which often undesirable side effect.

TDDS facilitate the passage of therapeutic quantities of drug substances through the skin and into the general circulation for their systemic effects.

In developing a transdermal delivery system, two criteria are considered: one is achieving adequate flux across the skin and the other is minimizing the lag time in skin permeation. One strategy overcoming this constraint is the incorporation of various chemical skin enhancers into the vehicle. Another strategy is a choice of an appropriate vehicle that corresponds to the drug being used for the dermal route of administration.

Concerning dermal application the microemulsions can interact with the stratum corneum changing structural rearrangement of its lipid layers and consequently increasing transdermal drug permeation and so act as penetration enhancer

1.1.1. Advantages of TDDS[3]

- Avoidance of first pass metabolism
- Avoidance of gastro intestinal incompatibility
- Predictable and extended duration of activity
- Minimizing undesirable side effects
- Provides utilization of drugs with short biological half life
- Narrow therapeutic window
- Improving physiological and pharmacological response
- Avoidance the fluctuation in drug levels
- Termination of therapy is easy at any point of time
- Greater patient compliance due to elimination of multiple dosing profile
- Ability to deliver drug more selectively to a specific site
- Provide suitability for self administration
- Enhance therapeutic efficacy

1.1.2. Limitations of TDDS

- Transdermal route administration is unsuitable for drugs that irritate or sensitize the skin
- Transdermal route cannot deliver in a pulsatile fashion

- Transdermal delivery is neither practical nor affordable when required to deliver large doses of drugs through skin
- Transdermal delivery cannot administer drugs that require high blood levels
- Drug of drug formulation may cause irritation or sensitization
- Not practical, when the drug is extensively metabolized in the skin and when molecular size is great enough to prevent the molecules from diffusing through the skin
- Not suitable for a drug, which doesn't possess a favourable, O/W partition coefficient
- The barrier functions of the skin of changes from one site to another on the same person, from person to person and with age

1.1.3. The Human Skin[4]

One highly successful alternative delivery method is the transdermal. Skin of an average adult body covers a surface of approximately $2m^2$ and receives about one-third of the blood circulating through the body. The deliver a drug into the body through transdermal layer of skin, it is necessary to understand about the skin.

The skin is the outer covering of the body. In humans, it is the largest organ of the integumentary system made up of multiple layers of epithelial tissues and guards the underlying muscles, bones, ligaments and internal organs. For the average adult human, the skin has a surface area between 1.5 to 2 m^2 (16.1-21.5 sq ft), most of it is between 2.3mm (0.10 inch) thick. The average square inch ($6.5cm^2$) of skin holds 650 sweat glands, 20 blood vessels, 60,000 melanocytes and more than a thousand nerve endings. It performs several essential functions. The adjective cutaneous literally means "of the skin" (from Latin cutis, skin).The different layers of the human skin is shown in fig 1.

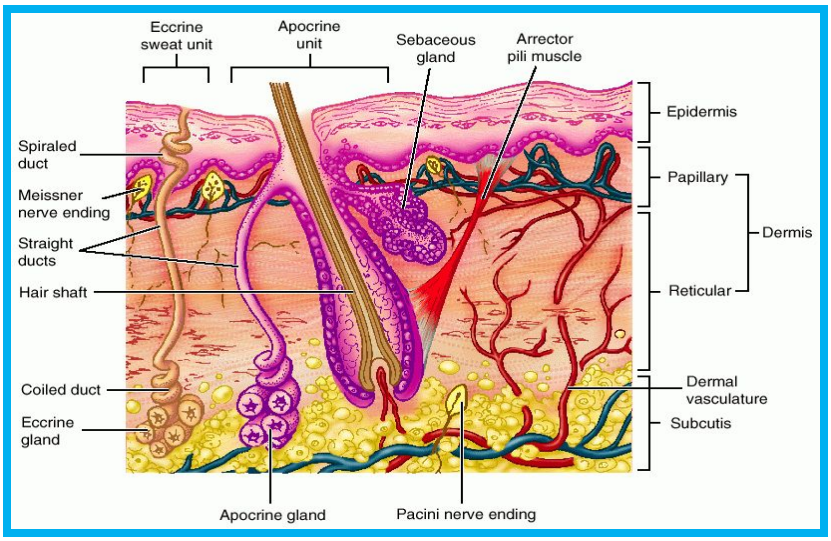


Fig 1: Anatomy of the Skin

1.1.4. Anatomy of the Skin

Structurally, the skin consists of two principle parts. The outer thinner portion, which is composed of epithelium, is called the epidermis. The epidermis is attached to the inner thicker, connective tissue part called the dermis. Beneath the dermis is a subcutaneous layer. This layer is also called the superficial fascia or hypodermis. It consists of areole and adipose tissues. Fibers from the dermis extend down into the subcutaneous layer and anchor the skin to it. The subcutaneous layer in turn attaches to underlying tissues and organs.

Skin layers:

Skin is composed of three primary layers.

- ♣ Epidermis
- ♣ Dermis
- ♣ Hypodermis (subcutaneous adipose layer).

Epidermis:

Epidermis, “epi’ coming from the Greek meaning “over” or “upon” is the outermost layer of the skin. It forms the water proof, protective wrap over the body’s surface and is made up of stratified squamous epithelium with an underlying basal lamina. It contains no blood vessels and cells in the deepest layer are nourished by diffusion from blood capillaries extending to the upper layers of the dermis.

Dermis:

Dermis is 3 to 5mm thick layer and is composed of a matrix of connective tissue, which contains blood vessels, lymph vessels and nerves. The cutaneous blood supply has essential function in regulation of body temperature. It also provides nutrients and oxygen to the skin while removing toxins and waste products.

Capillaries reach to within 0.2 mm of skin surface and provide sink conditions for most molecules penetrating the skin barrier. The blood supply thus keeps the dermal concentration of a permeant very low and the resulting concentration difference across the epidermis provides the essential concentration gradient for transdermal permeation. It contains hair follicles, sweat gland, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels.

Hypodermis:

The hypodermis or subcutaneous fat tissue supports the dermis and epidermis. It serves as a fat storage area. This layer helps to regulate temperature, provides nutritional support and mechanical protection. It carries principle blood vessels and nerves to skin and may contain sensory pressure organs. For transdermal drug delivery, drug has to penetrate through all these three layers and reach into systemic circulation while in case of topical drug delivery only penetration through stratum corneum is essential and then retention of drug in skin layers is desired. It consists of loose connective tissue and elastic. The main cell types are fibroblasts, macrophages and adipocytes.

1.1.5. Drug Delivery Routes Across Human Skin[6,7]

Drug molecules in contact with the skin surface can penetrate by three potential potential pathways: through the sweat ducts, via the hair follicles and sebaceous glands, (collectively called the shunt or appendageal route), or directly across the stratum corneum(Fig 2).

The relative importance of the shunt or appendageal route versus transport across the stratum corneum has been debated by scientists over the years(6-8) and is further complicated by the lack of a suitable experimental model to permit separation of the three pathways.

In vivo experiments tend to involve the use of hydrated skin or epidermal membranes so that appendages are closed by the swelling associated with hydration. Scheuplein and colleagues proposed that a follicular shunt route was responsible for the presteady-State permeation of polar molecules and flux of large polar molecules or ions that have difficulty diffusing across the intact stratum corneum.

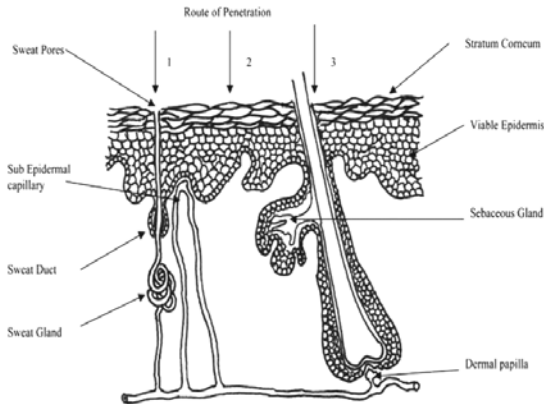


Fig 2: Routes of penetration

1. Through the sweat ducts; 2. Directly across the stratum corneum; 3.Via the hair follicles.

The stratum corneum consists of 10-15 layers of corneocytes and varies in thickness from approximately 10-15μm in the dry state to 40μm when hydrated. It comprises a multi-layered "brick and mortar" like structure of keratin-rich corneocytes (bricks) in an intercellular matrix (mortar) composed primarily of long chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulfate and sterol/wax esters. However it is important to view this model in the context that the corneocytes are not brick shaped but are polygonal, elongated and flat (0.2-1.5μm thick, 34-46 μm in diameter).

The intercellular lipid matrix is generated by keratinocytes in the mid to upper part of the stratum granulosum discharging their lamellar contents into the intercellular space. In the initial layers of the stratum this extruded material rearranges to form broad intercellular lipid lamellae, which then associate into lipid bilayers, with the hydrocarbon chains aligned and polar head groups dissolved in an aqueous layer Fig 3.

As a result of the stratum corneum lipid composition, the lipid phase behaviour is different from that of other biological membranes.the hydrocarbon chains are arranged into regions of crystalline,lamellar gel and lamellar liquid crystal phases thereby creating various domains within the lipid bilayers.The presence of intrinsic and extrinsic proteins,such as enzymes,may also affect the lamellar structure of the stratum corneum.

Water is an essential component of the stratum corneum,which acts as a plasticizer to prevent cracking of the stratum corneum and is also involved in the generation of natural moisturizing factor(NMF), which helps to maintain suppleness. In order to understand how the physicochemical propreties of the diffusing drug and vehicle influence permeation across the stratum corneum and thereby optimise delivery, it is essential to determine the predominant route of drug permeation with in the stratum corneum.

Traditionally it was thought that hydrophilic chemicals diffuse within the aqueous regions near the outer surface of intracellular keratin filaments

(intracellular or transcellular route) while lipophilic chemicals diffuse through the lipid matrix between the filaments (intracellular route)

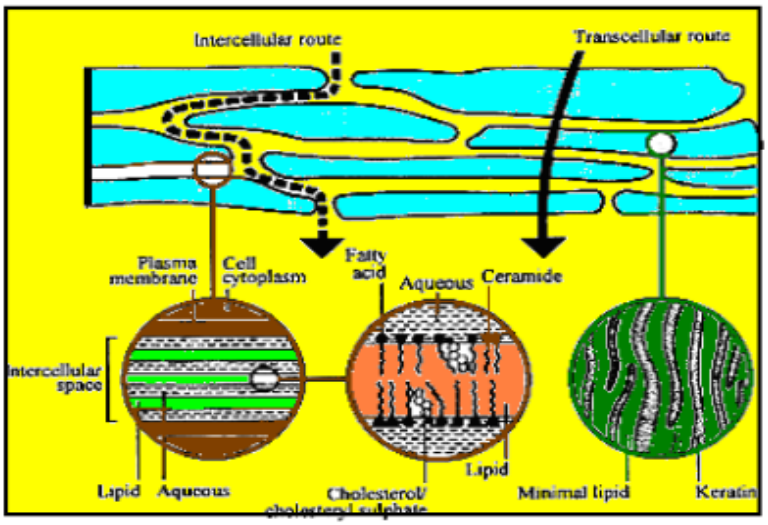


Fig 3: The stratum corneum and intercellular and transcellular routes of penetration

1.1.7 Factors Affecting Transdermal Permeation[7, 8]

Physicochemical factors:

A. Biological factors

i) Skin condition

Acids and alkalis, many solvents like chloroform, methanol damage the skin cells and promote penetration. Diseased state of patient alters the skin conditions. The intact skin is better barrier but the above mentioned conditions affect penetration.

ii) Skin age

The young skin is more permeable than older. Childrens are more sensitive for skin absorption of toxins. Thus, skin age is one of the factor affecting penetration of drug in TDDS.

iii) Blood supply

Changes in peripheral circulation can affect transdermal absorption.

iv) Regional skin site

Thickness of skin, nature of stratum corneum and density of appendages vary site to site. These factors affect significantly penetration.

v) Skin metabolism

Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. So skin metabolism determines efficacy of drug permeated through the skin.

vi) Species differences

The skin thickness, density of appendages and keratinization of skin vary species to species, so affects the penetration.

B. Physicochemical factors

i) Skin hydration

In contact with water the permeability of skin increases significantly. Hydration is most important factor increasing the permeation of skin. So use of humectant is done in transdermal delivery.

ii) Temperature and pH

The permeation of drug increases, ten folds with temperature variation. The diffusion coefficient decreases as temperature falls. Weak acids and weak bases dissociate depending on the pH and p_{ka} or p_{kb} values. The proportion of unionized drug determines the drug concentration in skin. Thus, temperature and pH are important factors affecting drug penetration.

iii) Diffusion coefficient

Penetration of drug depends on diffusion coefficient of drug. At a constant temperature the diffusion coefficient of drug depends on properties of drug, diffusion medium and interaction between them.

iv) Drug concentration

The flux is proportional to the concentration gradient across the barrier and concentration gradient will be higher if the concentration of drug will be more across the barrier.

v) Partition coefficient

The optimal partition coefficient (K) is required for good action. Drugs with high K are not ready to leave the lipid portion of skin. Also, drugs with low K will not be permeated.

vi) Molecular size and shape

Drug absorption is inversely related to molecular weight, small molecules penetrate faster than large ones. Ideal molecular properties for transdermal drug delivery.

1.2. Microemulsion[9, 10]

In 1943, Hour and Schulman visualized the existence of small emulsion-like structures by electron microscopy and subsequently coined the term "microemulsions". Microemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with a droplet size usually in the range of 10-100 nm. where as the diameter of droplets in a kinetically stable emulsion is >500 nm. Because the droplets are small, a microemulsion offers advantages as a carrier for drugs that are poorly soluble in water. These homogeneous systems, which can be prepared over a wide range of surfactant concentration and oil to water ratio, are all fluids of low viscosity.

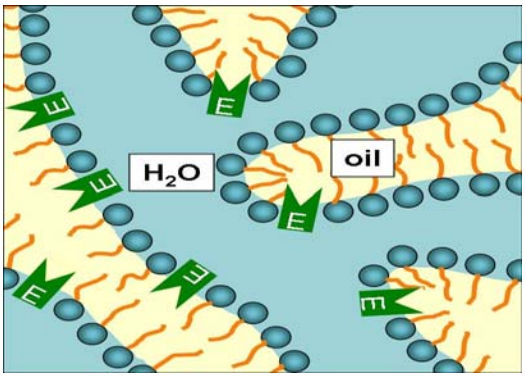


Fig 4: Microemulsion

1.2.1. Important Characteristics of Microemulsions[11, 12]

- Particle size 10-100 nm
- Thermodynamically stable (long shelf-life)
- Optically clear
- High surface area (high solubilization capacity)
- Small droplet size
- Enhanced drug solubilization
- Ease formation (zero interfacial tension and almost spontaneous formation)
- Ability to be sterilized by filtration
- Long-term stability
- High solubilization capacity for hydrophilic and lipophilic drugs
- Improved drug delivery

Table 1: Difference between Emulsion and Microemulsion

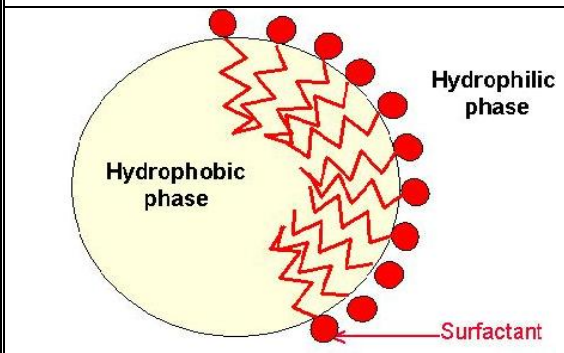
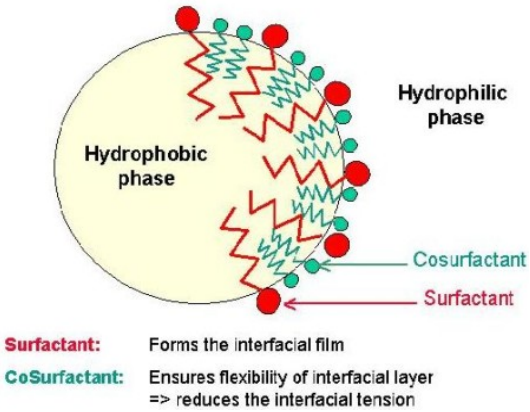
EMULSION	**MICROEMULSION**
	
EMULSION	**MICROEMULSION**
Emulsions consist of roughly spherical droplets of one phase dispersed into the other.	They constantly evolve between various structures ranging from droplet like swollen micelles to bi-continuous structure
Thermodynamically unstable (Kinetically Stable)	Thermodynamically stable (Long shelf-life)
Inefficient molecular packing	Efficient molecular packing
Direct oil/water contact at the interface	No direct oil/water contact at the interface
High interfacial tension	Ultra low interfacial tension
High viscosity	Low viscosity with Newtonian behavior
Droplet diameter: >500nm	10 – 100 nm
Cloudy colloidal system	Optically transparent(Isotropic)
They are lyophobic	They are on the borderline between lyophobic and lyophilic colloids
Require intense agitation for their formation	Generally obtained by gentle mixing of ingredients.
Ordinary emulsion droplets, however small exist as individual entities until coalesance or ostwald ripening occurs	Microemulsion droplet may disappear within a fraction of a second while another droplet forms spontaneously elsewhere in the system

Fig 5: Difference between Emulsion and Microemulsion

1.2.2. Microemulsion as Drug Delivery Systems[13]

1.2.3. Oral drug delivery

The most common method for drug delivery is through the oral route as it offers convenience and high patient compliance.

1.2.4. Parenteral drug delivery

Microemulsion systems intended for parenteral application have to be formulated using nontoxic and biocompatible ingredients. The oil in water microemulsion systems would be suitable to improve the solubility of poorly water soluble drug molecules whereas water in oil microemulsion systems would be best suited for optimizing the delivery of hydrophilic drug molecules that are susceptible to the harsh gastrointestinal condition

1.2.5. Ocular drug delivery

Aqueous solutions account for around 90% of the available ophthalmic formulations, mainly due to their simplicity and convenience However, extensive loss caused by rapid precorneal drainage and high tear turnover are among the main drawbacks associated with topical ocular drug delivery.

1.2.6. Topical drug delivery

1.2.7. Transdermal Drug Delivery

To the systemic circulation is one of the oldest routes that have been exploited using microemulsion systems.

1.2.8. Advantages of Microemulsion[14]

- Thermodynamically stable and require minimum energy for formation
- To increase the cutaneous absorption of both lipophilic and hydrophilic drugs when compared to conventional vehicles (emulsions, pure oils, aqueous solutions).
- Ease of preparation and high diffusion and absorption rates when compared to solvent without the surfactant system
- The formation of microemulsion is reversible. They may become unstable at low or high temperature but when the temperature to the stability range, the microemulsion reforms
- Drugs that are thermo-labile are easily incorporated without the risk of degradation
- Microemulsions act as supersolvent of drug. They can solubilize hydrophilic and lipophilic drugs including drugs that are relatively insoluble in both aqueous and hydrophobic solvents.
- This system is reckoned advantages because of its wide applications in colloidal drug delivery systems for the purpose of drug targeting and controlled release.
- A large amount of drug can be incorporated in the formulation due to the high solubilizing capacity that might increase thermodynamic activity towards the skin
- The surfactant and co surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers
- Low surface tension ensures good contact to the skin. Also, the dispersed phase can act as a reservoir making it possible to maintain an almost constant concentration gradient over the skin for a long time

1.2.9. Disadvantages of Microemulsion

- Use of large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
- Limited solubilizing capacity for high-melting substances
- The surfactant must be nontoxic for using pharmaceutical applications
- Microemulsion stability is influenced environmental parameters such as temperature and pH. These parameters change upon microemulsion delivery to patients

1.2.10. Techniques Used to Characterize Microemulsions and Related Systems[15]

The physicochemical and analytical techniques used to characterize microemulsion and related systems could be categorized into those used to:

- Elucidate the microstructure and monitor phase behavior changes
- Determine the droplet size of the disperse phase

The choice of a particular technique is limited by factors such as availability, feasibility, and the nature of the information sought. Pharmaceutical scientists are more focused on the usefulness of a particular microemulsion system for a drug delivery application and the influence of the microstructure on that, rather than on the fundamental understanding of aspects such as microstructure and phase behavior.

1.2.11. Structure of Microemulsions[16]

The mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon the proportions of the components. The flexibility of the surfactant film is an important factor in this regard. A flexible surfactant film will enable the existence of several different structures like droplet like shapes, aggregates and bicontinuous structures, and therefore broaden the range of microemulsion existence.

A very rigid surfactant film will not enable existence of bicontinuous structures which will impede the range of existence. Besides microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic

crystalline hexagonal or cubic phases, and lamellar structures depending on the ratio of the components.

The internal structure of a microemulsion vehicle is very important for the diffusivity of the phases, and thereby also for the diffusion of a drug in the respective phases. Researchers have been trying zealously to understand the complicated phase behaviour and the various microstructures encountered in the microemulsion systems.

1.2.12. Components of Microemulsion Formulations[17]

A large number of oils and surfactants are available which can be used as components of microemulsion systems but their toxicity, irritation potential and unclear mechanism of action limit their use. One must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that will result in mild and non-aggressive microemulsions. The emphasis is, therefore, on the use of generally regarded as Safe (GRAS) excipients.

Oil Phase:

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in increased negative curvature (and reduced effective HLB). Saturated (e.g. lauric, myristic and capric acid) and unsaturated fatty acids (e.g. oleic acid, linoleic acid and linolenic acid) have penetration enhancing property of their own and they have been studied since a long time. Fatty acid esters such as ethyl or methyl esters of lauric, myristic and oleic acid have also been employed as the oil phase. Lipophilic drugs are preferably solubilized in o/w microemulsions. The main criterion for selecting the oil phase is that the drug should have high solubility in it. This will minimize the volume of the formulation to deliver the therapeutic dose of the drug in an encapsulated form.

Surfactants:

The surfactant chosen must be able to lower the interfacial tension to a very small value which facilitates dispersion process during the preparation of the microemulsion and provide a flexible film that can readily deform around the droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region.

It is generally accepted that low HLB surfactants are favoured for the formulation of w/o microemulsion, whereas surfactants with high HLB (>12) are preferred for the formation of o/w microemulsion. Surfactants having HLB greater than 20 often require the presence of co-surfactants to reduce their effective HLB to a value within the range required for microemulsion formation.

Co-surfactants:

In most cases, single-chain surfactants alone are unable to reduce the o/w interfacial tension sufficiently to enable a microemulsion to form. The presence of co-surfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidizing groups (e.g. unsaturated bonds). Short to medium chain length alcohols (C3-C8) are commonly added as co-surfactants which further reduce the interfacial tension and increase the fluidity of the interface.

1.2.13. Method of preparation of Microemulsion[18]

1. Phase Titration Method

Microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. Microemulsions are formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. The understanding of their phase equilibria and demarcation of the phase boundaries are essential aspects of the study.

As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component .The region can be separated into w/o or o/w microemulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the metastable systems are not included.

2. Phase Inversion Method

Phase inversion of microemulsions occurs upon addition of excess of the dispersed phase or in response to temperature. During phase inversion drastic physical changes occur including changes in particle size that can affect drug release both *in vivo* and *in vitro*.

This method is referred to as phase inversion temperature (PIT) method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone. Additionally, a transition in the spontaneous radius of curvature can be obtained by changing the water volume fraction. By successively adding water into oil, initially water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a w/o microemulsion to an o/w microemulsion at the inversion locus.

Microemulsions can be prepared by controlled addition of lower alcohols (butanol, pentanol and hexanol) to milky emulsions to produce transparent solutions comprising dispersions of either water-in-oil (w/o) or oil-in-water (o/w) in nanometer or colloidal dispersions (~ 100 nm). The lower alcohols are called co-surfactants; they lower the interfacial tension between oil and water sufficiently low for almost spontaneous formation. The miscibility of oil, water and amphiphile (surfactant plus co-surfactant) depends on the overall composition which is system specific.

Microemulsions also have industrial applications, one of them being the synthesis of polymers. Microemulsion polymerization is a complex heterogeneous process where transport of monomers, free radicals and other species (such as

chain transfer agent, co-surfactant and inhibitors) between the aqueous and organic phases, takes place. Compared with other heterogeneous polymerization processes (suspension or emulsion) microemulsion polymerization is a more complicated system. Polymerization rate is controlled by monomer partitioning between the phases, particle nucleation, and adsorption and desorption of radicals. Particle stability is affected by the amount and type of surfactant and pH of dispersing medium.

1.2.14. Types of Microemulsions[19]

1. O/W Microemulsion

2. W/O Microemulsion

3. Bi-continuous Microemulsion

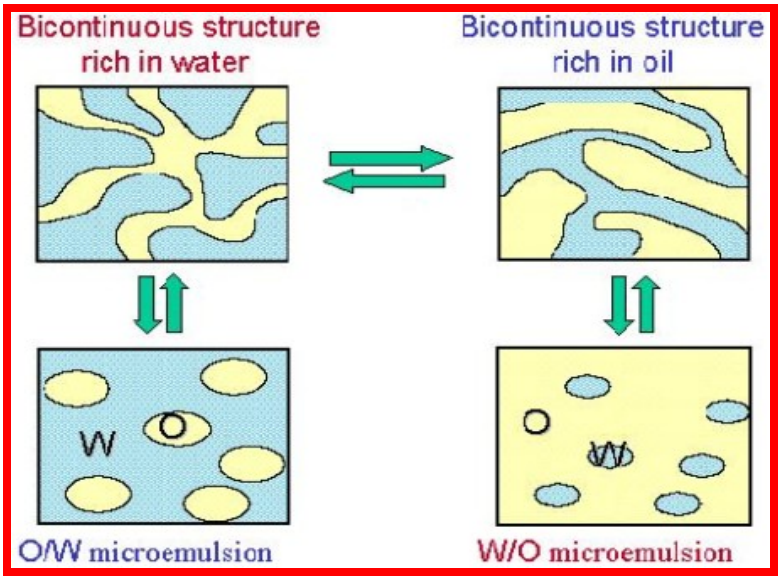


Fig 6: Types of Microemulsions

Oil in Water Microemulsions:

Oil droplets are dispersed in the continuous aqueous phase. The o/w systems are interesting because they enable a hydrophobic drug to be more soluble

in an aqueous based system, by solubilizing it in the internal oil droplets. Most drugs tend to favor small/medium molecular volume oils as opposed to hydrocarbon oils due to the polarity of the poorly water-soluble drugs.

Water in Oil Microemulsions:

Water droplets are dispersed in the continuous oil phase. Water-in-oil microemulsions are made up of droplets of water surrounded by an oil continuous phase. These are generally known as “reverse-micelles”, where the polar head groups of the surfactant are facing into the droplets of water, with the fatty acid tails facing into the oil phase.

Bi-continuous Microemulsions:

Micro domains of oil and water are interdispersed within the system. A bicontinuous microemulsion system both water and oil exist as a continuous phase. Irregular channels of oil and water are intertwined, resulting in what looks like a “sponge-phase”.

1.2.15. Factors affecting the Microemulsion Formulations[20]

The formation of microemulsion will depend on the following factors

a. Packing ratio:

The HLB of surfactant determines the type of microemulsion through its influence on molecular packing and film curvature. The analysis of film curvature for surfactant association's leadings to the formation of microemulsion.

b. Property of surfactant, oil phase and temperature:

The type of microemulsion depends on the nature of surfactant. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these group, which are a measure of the differential tendency of water to swell head group and oil to swell the tail area are important for specific formulation when estimating the surfactant HLB in a particular system.

When a high concentration of the surfactant is used or when the surfactant is in presence of salt, degree of dissociation of polar groups becomes lesser and resulting system may be w/o type.

Diluting with water may increase dissociation and leads to an o/w system. Ionic surfactants are strongly influenced by temperature. It mainly causes increased surfactant counter ion dissociation. The oil component also influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chains oils penetrate the lipophilic group region to a great extent and results in increased negative curvature.

Temperature is extremely important in determining the effective head group size of nonionic surfactants. At low temperature, they are hydrophilic and form normal o/w system. At higher temperature, they are lipophilic and form w/o systems. At an intermediate temperature, microemulsion coexists with excess water and oil phases and forms bicontinuous structure.

c. The chain length, type and nature of co-surfactant:

Alcohols are widely used as a co-surfactant in microemulsions. Addition of shorter chain co-surfactant gives positive curvature effect as alcohol swells the head region more than tail region so, it becomes more hydrophilic and o/w type is favoured, while longer chain co-surfactant favours w/o type w/o type by alcohol swelling more in chain region than head region.

Table 2: Marketed Product in Microemulsion Technique

S.no.	Drugs	Marketed products
1.	Cyclosporine	Neoral
2.	Ibuprofen	Solvium

1.3. Arthritis[21]

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation of the joints and may cause inflammation of other tissues in the body. The immune system consists of the cells and proteins in our bodies that fight infections. An autoimmune disease occurs when our immune system doesn't recognize part of our body and attacks it as if it were an invader such as a bacteria or virus.

In rheumatoid arthritis, the immune system targets synovial membrane and attacks it. The synovial membrane is secretes synovial fluid into the joint.

Synovial fluid is the joint fluid that lubricates and nourishes the joint. Other tissues can also be targeted by the immune system in rheumatoid arthritis, but the synovium, or synovial membrane, is generally the primary target. When the synovial membrane is attacked, it becomes inflamed (synovitis) and can thicken and erode. As the synovial membrane is destroyed, the synovial fluid fluid is also destroyed because it is not being secreted. The surrounding structures can also become involved leading to the joint deformities that can be seen in rheumatoid arthritis.

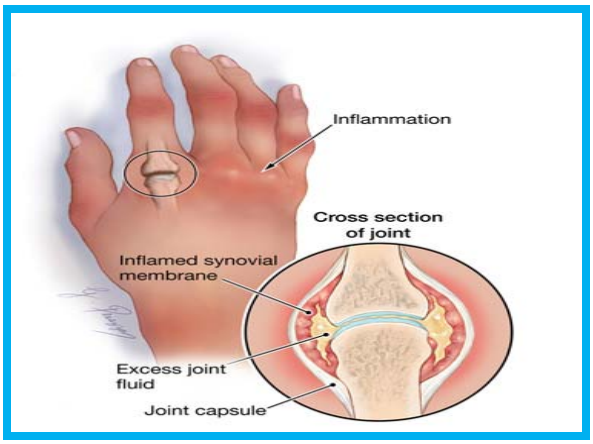


Fig 7: Rheumatoid Arthritis

1.3.1. Epidemiology of RA

- RA affects over 21 million people worldwide
- There are about 3 million people living with RA in Europe
- RA affects 3 times as many women as men
- Obesity
- Previous joint injury
- Ethnic background
- It can affect people of all ages but it is most common in the 30-50 age range

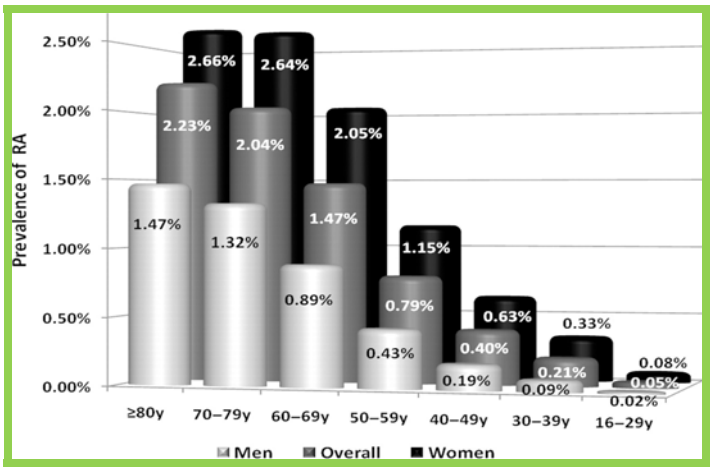


Fig 8: Prevalence of RA in Men, Overall and Women

1.3.2. Types of Arthritis[22]

Based on the causes of arthritis changes, several forms of arthritis can be named. A particular type of arthritis occurs in a particular age group and in a particular joint.

Table 3: Types of Arthritis

Arthritis	Age Group	Site
Osteoarthritis	Elderly	Knee, lower back, Fingers
Juvenile Rhumatoid arthritis	Childhood	Knee, hip
Septic arthritis	Childhood	Knee, hip
Rhumatoid arthritis	Young adults	Hip, Knuckles, Knee
Ankylosing spondylitis	Young adults	Lower back, Cheast
Psoriatic arthritis	Young adults	Knee
Traumatic arthritis	Any	Any (Commonly knee, hip, ankle)
Gout	Young adults	Big toe, knee

1.3.3. Clinical Features

- The stiffness is characteristically worse in the morning and improves during the day; its duration is a useful indicator of the activity of the disease. The stiffness may recur especially after strenuous active.
- The usual joints affected by rheumatoid arthritis are the metacarpophalangeal joints, the PIP joints, the wrists, knees, ankles and toes.
- Entrapment syndromes may occur especially carpal tunnel syndrome

- 20% of patients with RA will have subcutaneous nodules, usually seen over bony prominences but also observed in bursa and tendon sheaths; these nearly always occur in seropositive patients as do most other extra-articular manifestations
- Splenomegaly and lymphadenopathy can occur
- Low grade fever, anorexia, weight loss, fatigue and weakness can occur
- After months to years, deformities can occur; the most common are

  - Ulnar deviation of the fingers
  - Swan neck deformity, which is hyperextension of the distal interphageal joint and flexion of the proximal interphalangeal joint
  - Boutonniere deformity, which is flexion of the distal interphalangeal joint and extension of the proximal intraphalangeal joint valgus deformity of the knee.

1.3.4. Etiology for Arthritis[23]

There are two main groups of theories' regarding this disease

1. That it is non-infective in character
2. That it is infective

The former postulates that the disease can manifest itself in the absence of organisms that, it is essentially due to disordered body chemistry.

The latter holds that whether tissue changes resulting from non bacterial cause are present or not, it is essential that organisms be present locally.

Non infective character falls into three groups

1. Congential predisposition
2. Endocrine disturbance
3. Faulty alimentation

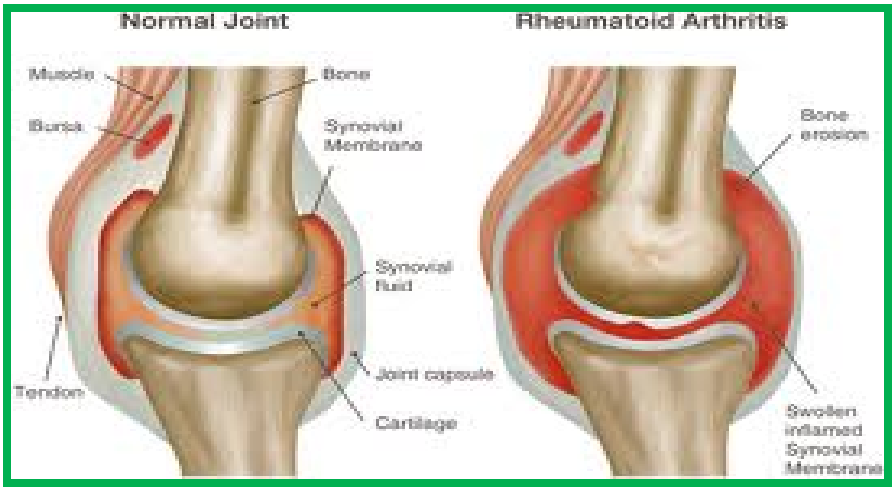


Fig 9: Difference between Normal joints and Rheumatoid Arthritis joints

Synovial macrophages and fibroblasts interact to perpetuate inflammation most of our knowledge of the inflammatory process and cellular infiltrate in the rheumatoid joint comes from the study of synovium in established, rather than early, disease, CD_4 T cells and monocytes-macrophages migrate into, and remain

in the synovial interaction of cellular adhesion molecules with counterligands expressed on extracellular matrix molecules (e.g., collagen, fibronectin).

Neutrophils, in contrast, are found almost exclusively in the synovial cavity (fluid) and only rarely in the synovial tissue. Their migration through the synovial interstitium and across the synovial lining into the joint cavity may reflect lack of expression of specific adhesion molecules for extracellular matrix constituents.

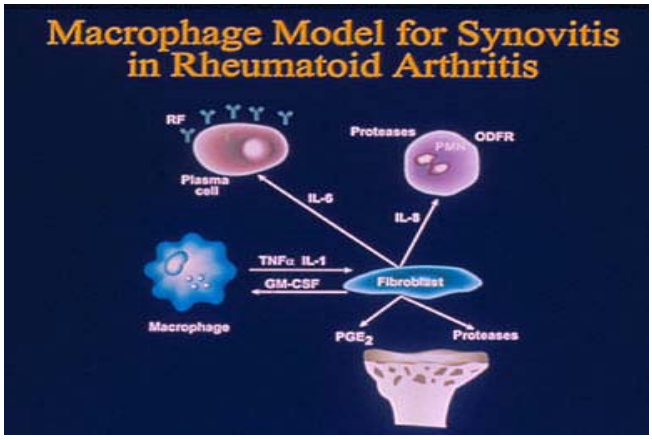


Fig 10: Pathogenesis of Rheumatoid Arthritis

According to the "T cell centric" theory of RA, activation of CD_4 cells would trigger and maintain the inflammatory process in the rheumatoid joint Interestingly, although large numbers of CD_4 cells persist in the synovium throughout the disease course, they appear to be inactive in the chronic phase of the disease. For example, expression of surface antigens (such as IL2 and transferrin receptors), and secretion of specific cytokines (e.g., IL2, IL4 and g-IFN), that are associated with an activated T cell state are very low.

Table 4: Cellular sources of synovial cytokines in RA

Products of T cells	IL-2 IL-3 IL-4 IL-6 IFNg TNFb GM-CSF

In contrast, cytokines known to be produced primarily by "effector" cells (macrophages) and connective tissue cells (fibroblasts) are expressed in abundance in RNA synovium and synovial fluid , as measured by ELISA or mRNA studies. These cytokines include IL1, IL6, IL8 and GM – CSF. According to the alternative theory (the "macrophage –fibroblast theory") of RA, these two cell types appear to be largely responsible for creating a self perpetuating state of chronic inflammation in which T cell participation may no longer be critical. In this scenario, the activated macrophage continuously secretes IL-1 and TNF which maintain the synovial fibroblast in an activated state.

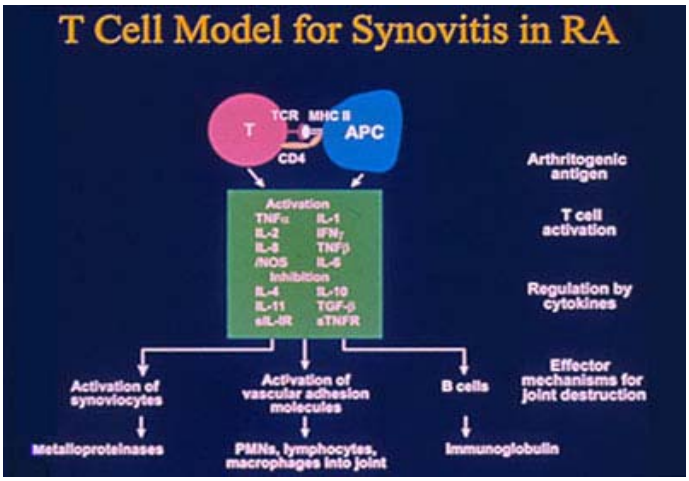


Fig 11: Synovitis in RA patients

The fibroblast, in turn, secretes large amounts of: a) cytokines – IL6, IL8 and GM-CSF; b) prostaglandins; c) protease enzymes. GM-CSF feeds back to promote the maturation of newly recruited monocytes to macrophages.IL-8 and IL-6 contribute to the recruitment and/or activation of yet other cell populations, while the prostaglandins and proteases act directly to erode and destroy nearby connective tissues such as bone and cartilage.

1.3.5. Inflammatory Mediators in RA

In addition to activating synovial cells to secrete inflammatory mediators, IL-1 and TNF also have profound systemic effects.

Table 5: Mediators in RA

Cellular	Systemic
• Upregulation of adhesion molecules • Costimulant for T cells • Induction of prostanoid synthesis • Induction of cytokine synthesis (IL-6, IL-8, GMCSF)	• Fever • Decreased appetite • Muscle wasting

Some of these systemic effects are mediated via the induction of IL-6 synthesis. Mature plasma cells that secrete rheumatoid factor are another prominent cellular component of rheumatoid synovium.

The stimulus for maturation of B cells to immunoglobulin-secreting plasma cells has classically been ascribed to CD_4 T cells; however, as already noted CD_4 T cells are not activated in the chronic phase of rheumatoid arthritis. IL-6, however, is a potent stimulus for maturation of B cells to plasma cells. Thus, synovial fibroblasts are likely providing the “T cell independent” stimulus for continuous plasma cell activation and rheumatoid factor production. IL-6 also suppresses albumin synthesis by the liver and stimulates acute phase protein synthesis. IL-6, therefore, contributes significantly to ESR elevation.

Table 6: Effects of IL-6

Effects of IL-6	
B cell maturation	Ig, rheumatoid factor , hypergammaglobulemia
Hepatocyte stimulus	Acute phase proteins (high ESR) Decreased albumin synthesis

Neutrophils are recruited in very large numbers to the rheumatoid cavity where they can be aspirated in the synovial fluid. Complement activation is not a prominent feature of RA. Therefore, C5a is unlikely to contribute significantly to the recruitment of neutrophils to the joint. IL-8, however, is also a potent and specific chemotactic stimulus for neutrophils.

Since synovial fibroblasts line the Joint cavity, their elaboration of this cytokine into the joint cavity is likely to explain the selective requirment of neutrophils to the synovial cavity. neutrophils in the synovial fluid are in an activated state, releasing oxygen-derived free radicals that depolymerize hyalurionic acid and inactivate endogenous inhibitors of proteases, thus promoting damage to the joint.

Prostaglandins and proteases are also secreted from synovial fibroblasts as the pannus invades contiguous bone and cartilage. PGE_2 resorbs bone and contributes to the radiographically demonstrable erosions at the site of synovial bone attachment. The proteases (collagenase, stromelysin and gelatinase) act enzymatically to degrade the collagen and proteoglycan matrix of bone and cartilage.

This destructive effect is further compounded by IL1 (and TNF) which suppresses synthesis of these matrix molecules. Thus, IL1 provides a “double insult” to connective tissue by both promoting its degradation (by inducing

synthesis of proteases) and preventing its repair (by suppressing synthesis of collagen and proteoglycans).

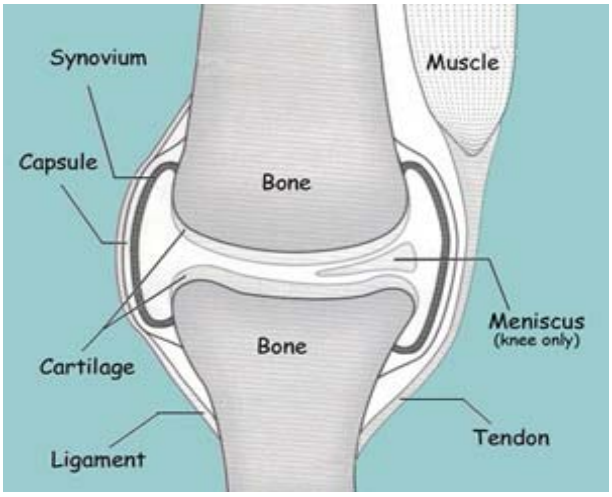


Fig 12: Normal view joint

A joint affected by arthritis loses its ability to provide smooth movement between the bones .this is because of the following changes taking place gradually over a period of time.

- Decrease in the amount of synovial fluid
- Wear and tear of articular cartilage
- Thickness and stiffness of synovium
- Stiffness and of the joint capsule

These changes can occur due to several reasons like ageing, autoimmune disorders (immune system destroys our own body), genetic disorders, traumatic incident (accident, fall, or blunt injury), infection, and so on.

The arthritic changes are generally permanent and cannot be reversed after a period of time. Hence, early recognition and treatment is the only way to prevent more damage.

1.3.6. Symptoms of Rheumatoid Arthritis

The hallmark symptom of RA is morning stiffness that lasts for at least an hour. (stiffness from osteoarthritis , for instance , usually clears up within half an hour).even after remaining motionless for a few moments , the body can stiffen. Movement becomes easier again after loosening up.

Fig 13: Symptoms of RA

Swelling and pain:

Swelling and pain in the joints must occurs for atleast six weeks before a diagnosis of RA is consider the inflamed joints usually swollen and often feel warm and “boggy” when touched .The pain often occurs symmetrically but may be more severe on one side of the body , depending on which hand the person uses more often(Fig 13).

Specific joints affected:

Although RA almost always develops in the wrists and knuckles , the knees and joints of the ball of the foot are often affected as well .Indeed ,many joints may be involved ,including those in the cervical spine , shoulders ,elbows, tips , temperomandibular joint (jaw) , and even joints between very small bones

in the inner ear . RA does not usually show up in the fingertips,where osteoarthritis is common , but joints at the base of the fingers are often painful .

Nodules:

Nodules can occur throughout the course of the disease. Rarely, nodules may become sore and infected, particularly if they are in locations where stress occurs, such as the ankles. On rare occasions, nodules can reflect the presence of rheumatoid vasculitis, a condition that can affect blood vessels in the lungs, kidneys, or other organs.

Fluid Buildup:

Fluid may accumulate, particularly in the ankles. In the joint sac behind the knee accumulates fluid and forms what is known as a Baker cyst. This cyst feels like a tumor and sometimes extends down the back of the calf causing pain. Baker cysts often develop in people who do not have RA.

Flu-Like Symptoms:

Symptoms such as fatigue, weight loss, and fever may accompany early RA, some people describe them as being similar to those of a cold or flu except, of course, RA symptoms can last for years.

Symptoms in children:

In children, juvenile RA, also known as still's disease, is usually preceded by high fever and shaking chills along with pain and swelling in many joints. A pink skin rash may be present.

1.3.7. Medications

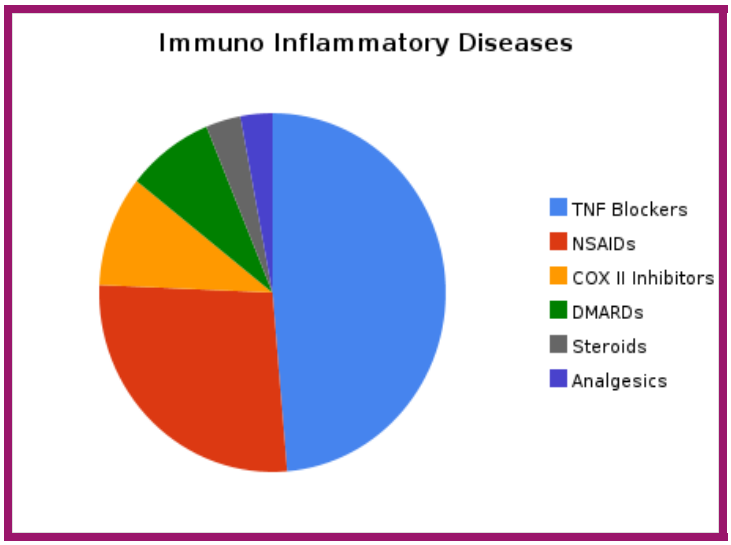


Fig 14: Medication of RA

Many drugs used to treat rheumatoid arthritis have potentially serious side effects. Doctors typically prescribe medications with the fewest side effects first. You may need stronger drugs or a combination of drugs as your disease progresses.

NSAIDS:

Enolic acid (Oxicam) derivatives

- Piroxicam
- Meloxicam
- Tenoxicam
- Droxicam
- Lornoxicam
- Isoxicam

Mechanism of Action[79]

Lornoxicam act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyzes the formation of prostaglandins and

thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2).

Prostaglandins act (among other things) as messenger molecules in the process of inflammation. NSAIDs have antipyretic activity and can be used to treat fever. Fever is caused by elevated levels of prostaglandin E2, which alters the firing rate of neurons within the hypothalamus, that control thermoregulation. Antipyretics work by inhibiting the enzyme COX, which causes the general inhibition of prostanoid biosynthesis (PGE2) within the hypothalamus.

PGE2 signals to the hypothalamus to increase the body's thermal set point. Ibuprofen has been shown to be more effective as an antipyretic than acetaminophen. Arachidonic acid is the precursor substrate for cyclooxygenase leading to the production of prostaglandins F, D & E.

1.3.8. Inflammation[25]

Inflammation (Latin, inflammo, "I ignite, set alight") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Fig 15). Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection.

Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.

Fig 15: Inflammation

1.3.9. Classification of Inflammation

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

1.3.10. Symptoms of Inflammation

- Redness
- Swollen joint that's tender and warm to the touch
- Joint pain
- Joint stiffness
- Loss of joint function

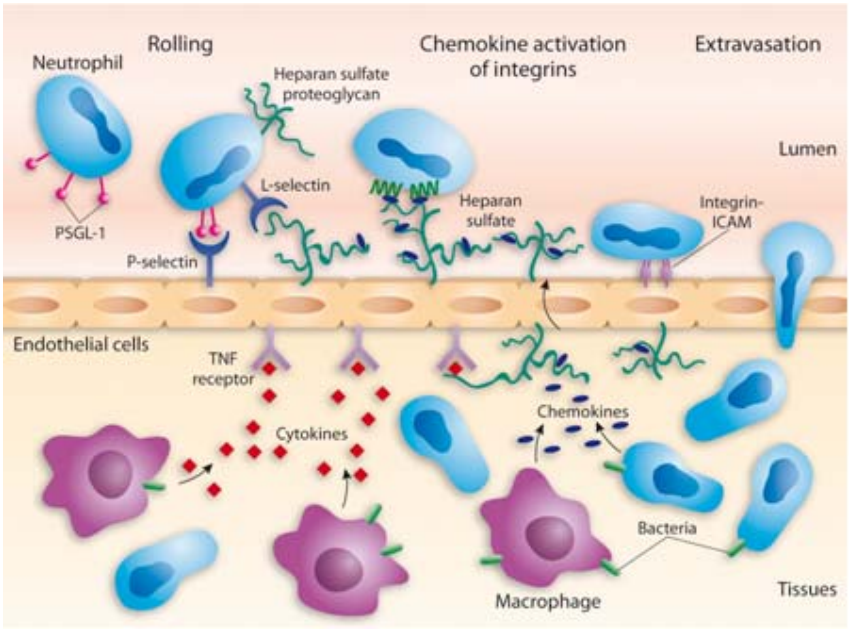


Fig 16: Mechanism of inlammation

1.3.11. Anti- inflammatory Effects[23]

Many mediators coordinate inflammatory and allergic reaction. While some are produced in response to specific stimuli (e.g. Histamine in allergic inflammation) there is considerable redundancy, and each facet of response – vasodilatation, increased vascular permeability, cell accumulation, etc-can be produced by several separate mechanisms.

The NSAIDS reduce mainly those component of the inflammatory and immune response in which prostaglandin, mainly derived from COX -2, play a significant part .These include:

- Vasodilatation
- Oedema (by an indirect action; the vasodilatation facilities and potentiating the action of mediators such as histamine that increase the permeability of postcapillary venules
- Pain ,again potentiating other mediators ,such as bradykinin

The NSAIDs suppress the pain, swelling and increasing blood flow associated with inflammation but have little or no action on the actual progress of the underlying chronic disease itself. As a class, they are generally without effect on other aspects of inflammation, such as leucocytes migration, lysosomal enzyme release and toxic oxygen radical production that contribute to tissue damage in chronic inflammatory conditions such as rheumatoid arthritis, vasculitis and nephritis.

2. LITERATURE REVIEW

According to the aim and objectives my research work I have reviewed the following literatures as support for the delivery system and diseases to carry out the project to its logical conclusion.

2.1 Literature review for Microemulsions

[26]**Jadupati *et al.*, 2011** developed the Insulin-loaded microemulsions for transdermal delivery using isopropyl myristate or oleic acid as the oil phase, Tween 80 as the surfactant, and isopropyl alcohol as the co-surfactant. The insulin permeation flux of microemulsions containing oleic acid through excised mouse skin and goat skin was comparatively greater than that of microemulsions containing isopropyl myristate. The insulin-loaded microemulsion containing 10% oleic acid, 38% aqueous phase, and 50% surfactant phase with 2% dimethyl sulfoxide (DMSO) as permeation enhancer showed maximum permeation flux (4.93 ± 0.12 g/cm^2/hour) through goat skin. The *in vitro* insulin permeation from these microemulsions was found to follow Zero order and the Korsmeyer-Peppas model (R^2 = 0.923 to 0.973) over a period of 24 hours.

[27]**Bhavika *et al.*, 2011** developed a microemulsion for enhancing the permeation of acyclovir using different penetration enhancer like DMSO, Menthol, and Eucalyptus oil. They concluded that 1% menthol incorporated as a penetration enhancer and it showed 10% increase in permeation rate of drug. The microemulsion system was investigated for viscosity, pH, refractive index, electrical conductivity, and permeation. The optimum formulation provided 76% drug release in 12 hr.

[28]**Xiaohui *et al.*, 2011** studied the microstructure characterization of microemulsion consisting of oleic acid, cremophor RH40, ethanol and water and investigate the influence of microstructure on the solubilization potential of the microemulsion to meloxicam. They concluded that the solubilization capacity of microemulsion is closely related with its microstructure. The solubilization of W/O microemulsion is the best, compared with other two (O/W, Bi continuous), where as the O/W is the weakest.

[29]**Ying *et al.*, 2011** investigated a microemulsion system for transdermal delivery of ligustrazine phosphate. Microemulsions containing isopropyl myristate, labrasol, plurol oleique® and water were investigated in pseudo-ternary phase diagrams. The optimized microemulsion with permeation flux of 41.01 μg/cm^2/h across rat skin *in vitro*, showed no obvious irritation on back skin of rabbits. The results indicated that the studied microemulsion system might be a promising vehicle for transdermal delivery of ligustrazine phosphate.

[30]**Manish *et al.*, 2010** formulated Glipizide Microemulsion by water titration method using oleic acid as oil phase, tween-80 as surfactant and propylene glycol as co-surfactant. Microemulsions were characterized for pH, viscosity, droplet size, in vitro release profile, ex-vivo diffusion study, irritancy tests, stability and in vivo evaluation. Five microemulsion formulations were prepared.Oleic acid is used as oil phase in 2, 4, 6, 8, 10% concentration of formulation content and then 6% (ME-3) obtained in clear form and have higher cumulative percent release than others. Non–ionic surfactant Tween-80 was selected because they are generally less toxic, produce less skin irritation. *In vivo* studies were carried out on wistar rats. The optimized microemulsion formulation was found to be o/w type emulsion and having mean particle size of 138±4.5 nm. The results indicated that the developed microemulsion systems, especially ME-3, may be promising vehicles for the transdermal delivery of glipizide.

[31]**Brajesh *et al.*, 2010** developed o/w microemulsion for transdermal delivery of poorly water soluble acyclovir by aqueous titration method. Characterization of microemulsion were done for droplet Shape and size, refractive index, pH, Viscosity, drug loading capacity. Oleic acid is a model skin permeation enhancer for transdermal drug carrier and poorly water soluble drug. The mean droplet size of microemulsion was found below 50 nm. The surface morphology of microemulsion was evaluated by TEM. They concluded that The drug loaded microemulsion oily phase droplets shapes were found to be spherical, the range of 41.91 to 52.79 nm. The viscosity values of all samples were low and relatively constant at 33.28 to 41.01 mP. All samples exhibited Newtonian flow behaviour, as expected from microemulsions.

[32]**Kalra *et al.*, 2010** developed aceclofenac microemulsion formulations to increase the effect, controlled permeation, increased drug solubilization capacity and to minimize oral side effects of drug. Investigated the potential of microemulsion gel formulation using nonirritating and pharmaceutically acceptable ingredients without using additional harmful permeation enhancers.

Permeation rate of aceclofenac evaluated by Keshary Chein diffusion cell which confirmed that drug can easily permeate through the skin due to small particle size of microemulsion. *In vivo* studies of drug molecule was done by anti-inflammatory (Acute) model and FCA (Chronic) model which indicated that effect of drug was enhanced by prepared microemulsion and microemulsion gel.

[33]**Anjali CH *et al.*, 2010** investigated the antibacterial activity of refined Sunflower oil, Tween 20, water microemulsion system. Pseudo-ternary phase diagram were constructed to obtain the concentration range of oil, surfactant and water. Three microemulsion formulations were prepared. The concentration of refined sunflower oil varied from 5 % to 15 %, the surfactant concentration varied from 10 % to 30 % and water concentration varied from 55 % to 85 %. When water concentration increased, conductivity of the microemulsion system increased upto 50 % of water concentration and after that become stable. When oil and surfactant concentration was increased, pH of the microemulsion system decreased.

[34]**Mrunali *et al.*, 2010** investigated the effect of vehicle on in vitro skin permeation of ketoconazole applied in o/w microemulsions. The optimized microemulsion formulation showed a highest permeation rate of 63.28 ± 4.93 μg cm^2 h. Candida Albicans were used as a model fungus to evaluate the antifungal activity of the best formula achieved. These results indicate that the studied microemulsion formulation might be a promising vehicle for future topical delivery of KTZ.

[35]**Fathy *et al.*, 2010** Determined the *in vitro* release of piroxicam in microemulsion formulations in different gel bases, such as, methyl cellulose (MC), carboxy methyl cellulose (CMC), hydroxypropyl methyl cellulose (HPMC), Carbopol 934, Carbopol 940, and Pluronic F-127 bases. The drug content of piroxicam, pH and viscosity was measured. The results showed that, the incorporation of piroxicam in microemulsion formulas could lead to enhancement of piroxicam release profiles by allowing constant and regular *in vitro* release. Three percent MC gel base showed the highest release of piroxicam-microemulsion after 180 min (97.70%) followed by 3% HPMC (94.0%) when compared to bases containing piroxicam alone.

[36]**Tian *et al.*, 2009** designed the Microemulsion System for Transdermal Drug Delivery of Amphotericin B using isopropyl myristate (IPM), Tween 80, isopropyl alcohol and water. The pseudo-ternary phase diagrams were constructed by the H_2O titration method and the structures of the microemulsion were determined by measuring electrical conductivities. The diffusion studies were performed *via* excised rabbit skin. At pH 5.14, amb microemulsion with *K*m 1:1, O/SC 1:9(mass ratio of oil phase to surfactant/cosurfactant blend), water content 64.6%, drug content (2.93±0.08) mg/ml, showed the maximum permeation rate (3.255±0.64) μg·cm−2·h−1, which is stable for a long time.

[37]**Rohit *et al.*, 2009** Prepared Five different Fluconozole formulations with various values of oil (5 – 25%), water (10 – 50%), and the mixture of surfactant and co-surfactant (at the ratio of 4) (45 – 65%). In-vitro permeation of fluconazole from the microemulsions was evaluated using Keshary Chien diffusion cells mounted with 0.45μ cellulose acetate membrane. The amount of drug permeated across was analyzed by HPLC and the droplet size and zeta potential of the microemulsions was characterized. The globule size ranged between 122 - 418nm. The permeability of optimised microemulsion formulation was increased approximately five folds than that of the marketed formulation. The results indicated that the microemulsion system studied would be a promising tool for enhancing the percutaneous delivery of fluconazole.

[38]**Zhai *et al*., 2009** formulated Penciclovir Microemulsion- based hydrogel for topical delivery. They compared the formulation with the commercial cream, microemulsion- based hydrogel and microemulsion significantly increased the permeation of the drug into both epidermis and dermis. After the characterization of the formulation they concluded that the microemulsion based hydrogel could be a promising vehicle for topical delivery of Penciclovir.

[39]**Arun *et al*., 2009** were formulated the two novel formulation of O/W microemulsion of ketoprofen for improving transdermal absorption. Formulations were prepared by constructing the pseudo-ternary phase diagrams using oleic acid, polysorbate-80, propylene glycol and water in different ratios and were gelled by incorporating cab-o-sil. The result of solubility study shown ketoprofen was highest in oleic acid, followed by ethyl oleate, isopropyl myristate, and isopropyl palmitate. Therefore, oleic acid was chose as oil phase for microemulsion. The oily mixtures of oleic acid, polysorbate-80 and propylene glycol led to increased in drug solubility. After extensive screening for physical characteristics and appearance, final ratios of surfactants- cosurfactants were decided. *In-vitro* diffusion study was carried out using artificial semipermeable membrane. The percentage of drug release across the membrane from marketed product, formulation-1 and formulation-2 were found to be 84.64%, and 90.20% respectively in 8 hrs

[40]**Mostafa *et al*., 2008** were formulated fluoxetine hydrochloride as a microemulsion form for transdermal delivery using various ratio of surfactant. Characterization of selected MEs was achieved by pH determination, centrifugation, particle size and viscosity measurements, determination of refractive index and morphology studies using TEM. Five ME formulation were prepared and Selected ME3 was tested for its ability to penetrate through rat skin. ME3 exhibited an optimum composition with regard to stability, pH value, viscosity, and droplet size and permeation rate for effective *in-vitro* delivery across an artificial cellulose membrane; it also exhibited good penetration ability

through rat skin confirming its feasibility as a transdermal delivery system for fluoxetine hydrochloride.

[41] **Gamal *et al.*, 2008** self microemulsifying and microemulsion systems for transdermal delivery of Indomethacin: Effect of phase transition was studied by in their study they investigated five formulations with fixed surfactant-oil ratio and increasing water content. They concluded that these formulation increased the transdermal drug flux compared to saturated drug solution in Phosphate buffered saline.

[42]**Ambade *et al.*, 2008** formulated and evaluated the Flurbiprofen Microemulsion using isopropyl myristate, Ethyl oleate as oils, aerosol OT as surfactant and sorbitan monooleate as cosurfactant. Thye investigated that the formulation by pass its gastrointestinal adverse effects. They concluded that flubriprofen from optimized microemulsion formulation was found to be more effective as compared to gel cream in inhibiting the caragenan induced rat paw edema at all time intervals.

[43]**Gamal *et al.*, 2008** developed Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion in which steroidal drug as oil, Tween 80 as surfactant, Ethanol, isopropanol, and propylene glycol as co surfactants. Pseudo ternary phase diagrams were constructed in the presence and absence of co surfactant. The presence of co-surfactant increased the transdermal drug flux compared to the co surfactant free formulation. Thus they concluded that the presence of co surfactants and its type can affect both the phase behavior and transdermal delivery potential of microemulsion.

[44]**Ke-Shu *et al.*, 2007** evaluated the transdermal permeability of pentoxifylline gel *in vitro* and *in vivo*. Gel was prepared with carbomer 934 as the base, and the Wistar rat was chosen as an animal model. The effects of percutaneous enhancers on the transdermal permeability of pentoxifylline gel were investigated by *in vitro* permeation experiments. Cumulative permeation at different times was determined by HPLC. 3% Azone and 5% propylene glycol were used as collaborative enhancers in an optimal formulation. Topical

concentrations at different times were measured by microdialysis *in vivo*. The transdermal process of pentoxifylline fits to a zero-order kinetic equation, and its release profile remains of the zero-order despite the addition of enhancers. In addition, a good *in-vitro-in-vivo* correlation was achieved.

[45]**Anna *et al.*, 2006** formulated a microemulsions as transdermal drug delivery vehicles by using oleic acid as permeation enhancers,L–α–phosphatidylcholine from egg yolk. Thus they concluded microemulsion were found as an effective vehicle of the solubilization of certain drugs and as protecting medium for the entrapped of drugs from degradation,hydrolysis and oxidation.It can provide prolonged release of the drug and prevent irritation.

[46]**Huabing *et al.*, 2006** were constructed microemulsion-base hydrogel formulation for topical delivery of ibuprofen ethyl oleate as oil phase, Tween 80 as surfactant, propylene glycol as cosurfactant. They formulated various microemulsion formulations were prepared and the abilities of various microemulsions to deliver ibuprofen through the skin were evaluated *in vitro*. Thus the studied microemulsion-based hydrogel showed a good stability indicated that the studied microemulsion-based hydrogel may be a promising vehicle for topical delivery.

[47]**Gupta *et al.*, 2005** designed and Testing of an effective oil-in-water microemulsion drug delivery system. They studied the new pseudoternary system of clove oil/Tween 20. Quarcetin drug was encapsulated in the vehicle and the hepatotoxicity of the vehicle with and without the drug was studied by estimating serum alkaline phosphates, glutamate pyruvate transaminase, urea and creatinine.

[48]**Haubing *et al.*, 2004** studied microemulsion systems for transdermal delivery of triptolide posessesing immunosuppressive, anti-fertility and anti cancer activities. They formulated the microemulsion using oleic acid as oil phase, tween 80 as surfactant and propylene glycol as a cosurfactant. After the chacterization of the formulated microemulsion they concluded that this microemulsion is a promising vehicle for the transdermal delivery of triptolide.

[49]**Fang *et al.*, 2004** made lipid- nano/submicron emulsions were made of isopropyl myristate, soya bean oil or coconut oil as the oil phase lecithin as the surfactant. They evaluated the physiochemical and *in vitro* permeation and *in vivo* topical application of the formulation. Thus they concluded the feasibility of this formulation for the topical delivery of the flubriprofen.

[50]**Baroli *et al.*, 2000** formulated and characterized the microemulsions for topical delivery of 8-methoxsalen using Tween-80, Span-80, 1, 2-Octanediol for the treatment of hyperprolioferative skin diseases. The formulations were characterized and thus they concluded that the microemulsion system may be appropriate vehicles for the topical delivery of 8- methoxsalen.

[51]**Mohammed *et al.*, 2000** investicated the aerosol-OT (AOT)/water/isopropyl myristate microemulsion were investigated as a carrier in transdermal drug delivery of tetracaine hydrochloride. Studied in vivo analgesic on rats and histopathological, irritation, and oxidative stress measurements on mice. The tetracaine hydrochloride encapsulated in AOT/water/isopropyl myristate showed an eightfold enhancement in the analgesic response of drug compared to the aqueous solution of the drug as measured by the tail-flick method. The local analgesic response time of tetracaine hydrochloride was dependent on the composition of the microemulsion. The local analgesic responses of tetracaine hydrochloride increased as the weight percentage of AOT and water increased up to a certain concentration in the microemulsion.

[52]**Shaun *et al.*, 1997** studied *Ex-vivo* permeation of indomethacin from a submicron emulsion through albino rabbit cornea. Solubility of the formulation was pH dependent and the penetration rate of indomethacin through excised rabbit eye cornea from the emulsion and from a marketed product were determined and compared using a novel mounted corneal diffusion assembly. They concluded that the apparent corneal permeability coefficient of indomethacin incorporated in the emulsion was 3.8 times greater than that of indomethacin in the marketed aqueous solution.

2.2. Literature review for Lornoxicam

[53]**Kavitha K *et al.*, 2011** developed and evaluated matrix-type transdermal Films containing Lornoxicam with different ratios of hydrophilic (Hydroxy Propyl methyl cellulose), and hydrophobic (Ethyl cellulose) polymers by the solvent evaporation technique. The prepared transdermal patches were evaluated for *in vitro* release, moisture absorption, moisture loss and mechanical properties. The diffusion studies were performed by using modified Franz diffusion cells. The drug release rate increased when the concentration of hydrophilic polymer was increased.

[54]**Parikh *et al.*, 2011** formulated and evaluated Fast dissolving tablets of Lornoxicam were prepared by sublimation method and effervescent technique using various excipients (Menthol, camphor, Anhydrous sodium bicarbonate and citric acid respectively in different concentrations).The concentration of subliming agents increased the disintegration time and wetting time of tablets was decreased in case of sublimation techniques.

[55]**Phani *et al.*, 2011** prepared sustained release matrix tablets of Lornoxicam. The tablets were formulated by wet granulation method by using 10%, 20%, 30%, and 40% Tamarind Seed Polysaccharide (TSP) as a natural binder and compared with maximum ratio of various binders (HPMC K4M, Sodium CMC, Guar Gum). Concluded that drug release pattern was mainly dependent on the type of polymer, 20% TSP binder release the drug which followed zero order kinetics.

[56] **Syed *et al.*, 2011** formulated and Evaluated Sustained Release Matrix Tablets of Lornoxicam. Various formulations were developed by using release rate controlling and gel forming polymers like HPMC (K4M, K15M, K100M) in single by direct compression method. Different proportion of HPMC was associated with decrease in the overall cumulative drug release rate. The higher viscosity polymer had been seen to inhibit the initial burst release of Lornoxicam.

[57]**Ganesh *et al.*, 2010** formulated chronomodulated drug delivery system of Lornoxicam to prolong its duration of action and thus reduce the frequency of usage and to minimize its irritant effect on the stomach. Microspheres of Lornoxicam were prepared by using polymers like Gelatin, Na CMC and Chitosan.

[58]**Sandeep *et al.*, 2010** studied design and evaluation of mucoadhesive bi-layered buccal patches of lornoxicam. Bi-laminated films composed of drug (Lornoxicam) and chitosan, with (HPMC) and backing layer (ethyl cellulose). Films were fabricated by solvent casting technique.To increase HPMC concentration and increase folding endurance of the films. The swelling index increases with increase in HPMC concentration.

[59]**Bhavsar *et al.*, 2010** development and validation of uv-spectrophotometric Method for simultaneous estimation of paracetamol and Lornoxicam in bulk and tablet dosage form and concludes that simultaneous equation method is simple, precise, and accurate for the rapid determination of PARA and LOX in combined tablet dosage forms and this method may be successfully applied in control laboratories for their determination in combined dosage form.

[60]**Lakshmi *et al.*, 2010** studied simultaneous spetrometric estimation of paracetamol and lornoxicam in tablet dosage form and concludes that simultaneous and absorbance ratio methods were validated for linearity, accuracy and precision.

[61]**Rajesh *et al.*, 2009** studied lornoxicam is extremely bitter in taste to develop a bitterless oral disintegrating tablet of lornoxicam, masking was done by Eudragit EPO in different ratios, In this superdisintegrants were used while preparing tablets and concluded that tablets shown good taste and disintegrantion in oral cavity.

[62]**Berry *et al*., 1992** studied a double blind, multicentre, placebo controlled trial of lornoxicam in patients with osteoarthritis of the hip and knee and concluded that the lornoxicam was generally well tolerated, though some gastrointestinal side effects were seen as has been reported with other NSAIDs and no evidence of drug toxicity.

2.3. Literature review for *In vivo* studies

[63]**Fathy I *et al*., 2011** designed to compare the anti-inflammatory and analgesic effects of piroxicam in microemulsion formulation incorporated in different pharmaceutical gel bases to the commercial product (Feldene Gel®). The anti-inflammatory activity of the tested piroxicam formulations was evaluated using right hind paw oedema size of rats induced by carrageenan injection, while the analgesic effect was evaluated using Hot Plate method applied on mice. produced a maximum percent oedema inhibition after 1 hr (75.7% and 76.90%), respectively, while the analgesic effect of the same previous formulae produce a maximum increase in reaction time (analgesic effect) but after 1.5 hr (62.8% and 65.8% seconds) respectively, and then this analgesic effect was continued significantly for 3 hrs. indicating that the tested piroxicam formulations exhibited good and acceptable pharmacological effects (i.e. anti-inflammatory and analgesic effects) in comparison to the commercial product (Feldene Gel®).

[64]**Nahla *et al*., 2011** investigated the potential of microemulsion formulations for transdermal delivery of indomethacin were prepared by the spontaneous emulsification method, and characterized for morphology, droplet size, and rheological characteristics. The *ex vivo* skin permeation studies were performed using Franz diffusion cell with rabbit skin as a permeation membrane. The anti-inflammatory effects of microemulsion formulations showed a significant increase in percent edema inhibition value after 4 hours. It is promising that the concentration of indomethacin used to treat relative skin inflammatory conditions could be decreased due to the high permeation ability of indomethacin microemulsion and side effects of indomethacin might be reduced. Thus, this study suggests that, transdermal administration may be considered as an

alternative noninvasive method for indomethacin delivery to achieve rapid onset of its pharmacological effects.

[65]**Ashutosh *et al.*, 2008** Synthesis, Characterization and Pharmacological Evaluation of Amide Prodrugs of Flurbiprofen.They suggested retard the adverse effects of gastrointestinal origin. They subjected the synthessised prodrugs for bioavailability studies, analgesic, anti-inflammatory activities and ulcero genic index. Thus they concluded that these prodrugs can be considered for sustained release purpose.

[66]**Buritova J *et al.*, 1998** evaluated the anti-inflammatory and analgesic effects of Lornoxicam in the carrageenan model of inflammatory nociception. They concluded that Lornoxicam reduces the carrageenan- evoked odema at low doses of lornoxicam.

3. AIM AND OBJECTIVE

The aim of my present study is to Develop and Evaluate Microemulsion for Transdermal Delivery of Lornoxicam by using oleic acid at different ratios for the treatment of Rheumatoid Arthritis and inflammation.

Design of Microemulsion formulation for transdermal delivery of drugs, having the potential to increase the solubility of poorly water soluble drugs

- ♣ To avoid the first pass metabolism and there is a potential to deliver the drug in a controlled manner
- ♣ To have the possibility of immediate withdrawal of the treatment if necessary
- ♣ To minimize the adverse effect on the g.i.t like mild dyspepsia and heartburn to ulceration and hemorrhage
- ♣ Reduction of dosing frequency due to longer duration of action
- ♣ To improve patient compliance
- ♣ To achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation
- ♣ To provide sustained release drug for longer periods of time due to short half life
- ♣ To delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilization capacity and long shelf-life

4. PLAN OF WORK

- Title selection
- Literature Review
- Selection of drug and Excipients
- Preformulation Study:
    a. Description
    b. Melting Point
    c. Solubility
    d. Hygroscopic Nature
    e. Identification of the drug
        - UV absorption maxima
        - FTIR studies
    f. Drug- Excipient compatibility studies by FTIR
- Selection of Oil, Surfactant and Co-Surfactant
- Trial Formulations
- Optimization of Microemulsions
- Selection of best formulation
- Characterization of Microemulsions
    a. Optical Transparency
    b. Determination of pH
    c. Viscosity Measurements
    d. Mechanical Stress Study
    e. Drug Content Analysis

e. Particle Shape and Surface Morphology by

1. Transmission Electron microscopy (TEM)
2. Atomic Force Microscopy(AFM)

f. Particle size Measurement by

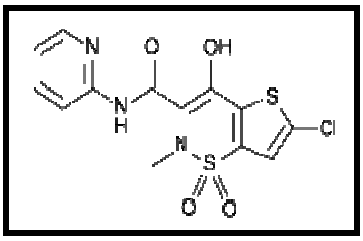
1. Particle size analyzer

- Evaluation Studies:
  - *In-Vitro* Skin permeation study
  - *In -Vivo* study (Anti-inflammatory activity)
- Release kinetics
- Stability testing as per ICH guidelines
- Results and Discussion
- Conclusion

5. DRUG AND EXCIPIENT PROFILE

5.1. LORNOXICAM[66, 67]

Chemical Structure:



Category	**:** Rheumatoid arthritis and osteoarthritis Anti-inflammatory, antipyretic, and analgesic activities.
Brand name	**:** Xafon, Lorcam
Chemical name	**:** 6-Chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H- thien [2, 3,-e]-1, 2-thiazine-3-carboxamide 1, 1- dioxide.
Empirical Formula	**:** $C_{13}H_{10}CLN_3O_4S_2$
Molecular Weight	**:** 371.82g/mol
Description:	
Colour	: Yellow crystalline powder
Taste	: weakly acidic drug
Solubility	**:** soluble – 0.1N hydrochloric acid and pH 7.4 slightly soluble - water and methanol
PK_a	: PKa of the lornoxicam is 24.7
Storage	: Protected from light and moisture
Bioavailability	: 90-100%
Metabolism	: CYP2C9
Half life	: 4-5 hours
Excretion	: 2/3hepatic, 1/3renal

Pharmacological actions[68]:

Lornoxicam inhibits prostoglantin biosynthesis by blocking the enzyme cyclooxygenase. Lornoxicam inhibits both isoforms in the same concentration range, that is, COX-1 inhibition: COX-2 inhibition = 1. Lornoxicam synovial fluid: plasma AUC ratio is 0.5, after administration of 4 mg twice daily.

Interactions:

Drug Interaction [69]:

Table 7: Drug Interaction with Lornoxicam

Drugs	Drug interactions
Blood pressure medication	Reduced pressure lowering effect
Alcohol	Potential gastrointestinal symptoms
Warfarin	Risk of bleeding
Asthma medication	Reduced effect of asthma medication; risk of asthma attack
Cardiac glycosides, digoxin	Risk that heart failure may be more severe
Corticosteroids	potential gastrointestinal symptoms
Cholestyramine	Reduced effect of Lornoxicam
Ciclosporin	Potential Lornoxicam toxicity
Methotrexate	Potential methotrexate toxicity
Diuretics	Potential risk of kidney damage
Baclofen	Potential baclofen toxicity

Disease interactions[70]:

Consult your doctor before using this drug if you have asthma, a stomach ulcer, a bleeding disorder, you are taking blood thinning medication or if you are allergic to aspirin or any other medication.

Recommended dosage:

Adults: 8-16mg/day in 2-3 divided doses.

Contraindications[71]:

The drug is contraindicated in patients that must not take other NSAID'S possible reasons including salicylate sensitivity, gastrointestinal bleeding and bleeding disorder and severe impairment of heart, liver or kidney function. Lornoxicam is not recommended and is contraindicated during the last third of pregnancy.

Adverse effect[72]:

Lornoxicam has side effects similar to other NSAID'S most commonly mild once like gastrointestinal disorders (nausea and diarrhea) and headache. Severe but seldom side effects include bleeding, bronchospasms and the extremely rare Stevens – Johnson syndrome.

Warning:

Do not use lornoxicam in children under the age of 18. According to Nycomed.com, in the event of an overdose, the user will experience more severe forms of the side effects. In the event of a real or suspected overdoses, contact your doctor immediately. Never use lornoxicam if you have a history of liver conditions, ulcers or blood coagulation problems.

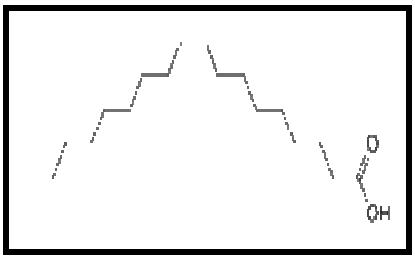
Common Side effects:

The most common side effects reported with the regular use of the tablet form of lornoxicam include dizziness, headache, stomach pain, upset stomach, diarrhea, nausea, vomiting and indigestion. As an injection, user most commonly report headache, flushing, insomnia and redness and irritation at the injection spot. Though common and less serious than other effects, these side effects are cause for concern if they worsen or occur regularly. In such an event, contact your doctor.

5.2. EXCIPIENT PROFILE

5.2.1 Oleic Acid[73, 74, 75]

Chemical structure:



Molecular Formula	**:**	$C_{18}H_{34}O_2$
Synonyms	**:**	Ethyl 9-octadecenoate, Kessco EO
Chemical name	**:**	(Z)-9-Octadecenoic acid, ethyl ester
Non Proprietary Name		
BP, USP	:	Oleic acid
Ph Eur	:	Acidum oleicum
Molecular Weight	**:**	282.4614 g/mol
Functional Category	**:**	Skin penetrant, Emulsifying agent
Description	**:**	Pale yellow or brownish yellow oily liquid with lard-like odor
Solubility	**:**	Soluble in acetone, chloroform, ether, Insoluble in water.

Pharmacopeial Specifications:

Specific gravity	:	0.866–0.874
Viscosity	:	5.15 mPa s
Refractive index	:	1.443–1.450
Acid value	:	Þ0.5 =Þ0.5
Iodine value	:	75–90, 75–85

Specific Properties:

Melting point	**:**	13-14 °C (286 K)
Flash point	**:**	175.3°C
Freezing point	**:**	˜×-×32°C
Moisture content	**:**	0.08% (at 20°C and 52%RH)
Surface tension	**:**	32.3 dynes/cm at 25°C
Viscosity (dynamic)	**:**	3.9 cP at 25°C
Viscosity (kinematic)	**:**	4.6 cSt at 25°C

Pharmaceutical Application:

- Oleic acid is used as an emulsifying agent in foods and topical pharmaceutical formulations. It has been used as a penetration enhancer in transdermal formulations, to improve the bioavailability of poorly water-soluble drugs in tablet formulations, and as part of a vehicle in soft gelatin capsules.
- Oleic acid has been reported to act as an ileal 'break' that slows down the transit of luminal contents through the distal portion of the small bowel.
- Oleic acid is used as a vehicle for certain parenteral preparations which are to be administrated by the intramuscular route
- It has also been used as a solvent for drugs formulated as biodegradable capsules for subdermal implantation and in the preparation of microemulsions containing cyclosporine
- Oleic acid labeled with ^{131}I and ^{3}H used in medical imaging

Safety :

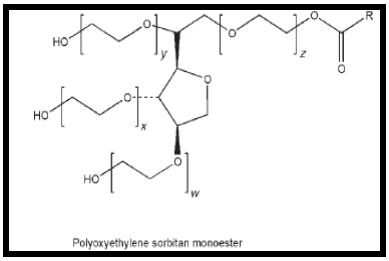
- Ethyl oleate is generally considered to be of low toxicity but ingestion should be avoided.
- Ethyl oleate has been found to cause minimal tissue irritation. No reports of intramuscular irritation during use have been recorded.

Stability & Storage

Oleic acid should be stored in a cool, dry place in a small, well-filled, well-closed container, protected from light.

5.2.2. Tween-20[76]

Chemical Structure:



$w+x+y+z=$**20**

R=Fatty acids

Synonyms	**:**	Armotan PML 20; Tween 20.
Chemical Name	**:**	Polyoxyethylene 20 sorbitan monolaurate
Non-Proprietary Name	**:**	Polysorbate 20
Molecular formulae	**:**	$C_{58}H_{114}O_{26}$.
Molecular Weight	**:**	1128g/mol
Functional Category	**:**	**N**onionic surfactant; suspending agent; wetting agent.

Description:

Colour : Yellow oily liquid at 25^0C

Odour and Taste : Polysorbates have a characteristic odor and a warm, bitter taste.

Applications in Pharmaceutical Formulation or Technology:

- Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions.
- Used as a solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins,
- Wetting agents in the formulation of oral and parenteral suspensions.

Table 8: Pharmaceutical application of Tween-20

Funtions	**Dosage form**	**Concentration (%)**
Emulsifying agent	O/W emulsions	1–15
	Combination with hydrophilic emulsifiers in O/W emulsions	1-10
	Ointments	1-10
Solubilizing agent	Poorly soluble active constituents in lipophilic bases	1-10
Wetting agent	Insoluble active constituents in lipophilic bases	0.1-3

Typical properties:

Acid value : 2.0%

Specific gravity **:** 1.1 at 25°C

Viscosity **:** 400 mPa s

Surface tension **:** 34.7(mN/m) at 20°C

Incompatibilities:

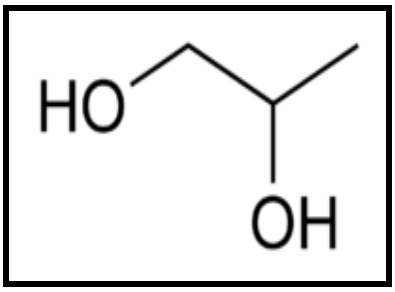
Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials.

Stability and Storage Conditions:

- Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation.
- Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.
- Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry.

5.2.2 Propylene Glycol[77]

Chemical Structure:



Molecular Formula	**:** $C_3H_5o_2$
Synonyms	**:** propylene glycol, α-propylene glycol, 1, 2-propanediol,
Chemical Name	**:** propane-1, 2-diol
Nonproprietary Names (BP)	**:** Propylene glycol
Molecular Weight	**:** 76.09g/mol
Funtional Category	**:** Antimicrobial preservative; disinfectant; humectants
Description	**: 1.**A clear and colorless **2.** Viscous and practically odorless **3**. Sweet, slightly acrid taste **4.** Resembling glycerol
Typical Properties:	
Boiling point	**:** 188°C
Flash point	**:** 99°C
Freezing point	: -59°C
Surface tension	**:** 40.1 dyne/ cm at 25°C
Density	**:** 1.038 g/cm3 at 20°C
Specific heat	**:** 2.47 J/g (0.590 cal/g) at 20°C
Vapor density (relative)	**:** 2.62 (air = 1)
Vapor pressure	**:** 9.33 Pa (0.07 mmHg) at 20°C
Viscosity (dynamic)	**:** 58.1 mPa s (58.1 cP) at 20°C

Solubility:

Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble at 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils, but will dissolve some essential oils.

Safety:

Propylene glycol is used in a wide variety of pharmaceutical formulations and is generally regarded as a relatively nontoxic material.

Stability and Storage Conditions:

At cool temperatures, propylene glycol is stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid.

Propylene glycol is chemicallystable when mixed with ethanol (95%), glycerin, or water; aqueous solutions may be sterilized by autoclaving. Propylene glycol is hygroscopic and should be stored in a well-closed container, protected from light, in a cool, dry place.

Applications in pharmaceutical Formulation or Technology:

- Propylene glycol has become widely used as a solvent, extractant, and preservative in a variety of parenteral and nonparenteral formulations.
- It is a better general solvent than glycerin and dissolves a wide variety of meterials, such as corticosteroids, phenols, Sulfa drugs, barbiturates, Vitamins (A and D), most alkaloids, and many local anesthetics.
- As an antiseptic it is similar to ethanol, and against molds it is similar to glycerin and only slightly less effective than ethanol.

Table 9: Pharmaceutical application of Propylene Glycol

Funtion	Dosage form	Concentration (%)
Solvent or co-solvent	Oral solutions	10-25
	Parenterals	10-60
	Topical preparations	5-80
	Aerosol solutions	10-30
Humectant	Topicals	15
Preservative	Solutions, semisolids	15-30

6. MATERIALS AND EQUIPMENTS

Table 10: List of Materials Used

S.no	Category	Materials	Manufacturer
1	Drug	Lornoxicam	Premier drug house, kandivali (west), Mumbai.
2	Oil	Oleic acid	Microfine chemicals,India
3	Oil	Olive oil	Scientific Chemicals, Chennai
4	Oil	Castor oil	Hi Pure Fine Chem Industries, Chennai.
5	Oil	Isopropyl myristate	Nice Chemicals Private Limited, Chennai
6	Oil	Isopropyl palmitate	Nice Chemicals Private Limited, Chennai
7	Surfactant	Tween-20	Sigma aldrich chemie, Germany
8	Surfactant	Span-20	Sigma aldrich chemie, Germany
9	Co- surfactant	Propylene glycol	Nice chemicals private limited, Chennai.
10	Co- surfactant	Isopropyl alcohol	Nice Chemicals Private Limited, Chennai

S.no	Category	Materials	Manufacturer
11	Co- surfactant	N-butanol	Scientific Chemicals, Chennai
12	Reagent	Potassium dihydrogen ortho phosphate	Microfine chemicals, New Delhi.
13	Reagent	Sodium hydroxide	Microfine chemicals, New Delhi.
14	Reagent	Hydrochloric acid	Microfine chemicals, New delhi.
15	Reagent	Methanol	Ranken, India.

Table 11: List of Equipments Used

S.no	Equipments	Manufacturer	Use
1	UV-Visible double beam spectrophotometer	Shimadzu UV 1700 (Pharmaspec)	To measure the absorbance of the sample
2	Electronic Balance	Sortorius Single Pan	For weighing purpose
3	Magnetic Stirrer	Remi equipment, Mumbai.	Microemulsions preparation
4	pH meter	Elico L 1120	To measure the pH of the solution
5	Brookfield Viscometer	LVII model	To measure the viscosity
6	Cooling centrifuge	Remi	Phase separation study
7	FTIR	Perkin Elmer	Compatibility study
8	Optical microscope	Nikon U.S	To identify the formulations
9	Digital camera	Sony Cybershot, 12.1 mega pixels	For pictures
10	Particle size Analyser	Microtrac-Blue wave, U.S	To measure the particle size

S.no	Equipments	Manufacturer	Use
11	AFM	Commercial Nanoscope III Digital Instruments, Veeco,	Surface morphology and the particle size
12	TEM	Topcon, Paramus, NJ	Morphology and shape
13	Digital plethysmometer	Orchid scientifics, India.	Anti-inflammatory activity
14	Graph pad software	Sandiego, CA	Kinetics of drug release, Student't-test
15	Environmental stability testing chamber	Heco Environment Chamber	For stability studies

7. PREFORMULATION STUDIES

Preformulation may be described as a stage of development process during which the researches characterize the physical, chemical and mechanical properties of the drug substance to form effective, stable and safe dosage form. Hence, preformulation studies are essential to characterize the drug for proper designing of the drug delivery system. The preformulation studies which were performing in this project include,

- Description
- Melting point
- Solubility
- Hygroscopic Nature
- Identification of drug sample
- Drug – excipient compatibility studies

7.1. Description

Organoleptic characters of drug was observed and recorded by using descriptive terminology.

7.2. Melting point

Capillary tube, which is sealed at one end is charged with sufficient amount of dry powder to form a column in the bottom of the tube 2.5mm to 3.5mm, and packed down as closely as possible by moderate tapping on a solid surface. The apparatus is operated according to the standard operating procedure. The block is heated until the temperature is about 30^{o} C below the expected melting point. The capillary tube is inserted into the heating block, and the heating is continued at a rate of temperature increased of about 1^{o} C to 2^{o} C per minute until melting is completed.

The temperature at which the detector signal first leaves its initial value is defined as the beginning of melting, and the temperature at which the detector signal reaches its final value is defined as the end of melting, or the melting point. The two temperatures fall within the limits of the melting range.

7.3. Solubility Studies

The spontaneous interaction of two or more substance to form a homogenous molecular dispersion is called as solubility.

10 mg of drug was a suspended separately in 10 ml of different solvents at room temperature in tightly closed tubes and shaken. The solubility profiles of two drugs in various solvents are shown in the table (12).

Table 12: Solubility Profile I.P. 1996

Descriptive term	Parts of solvent required for 1 part of solute.
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10, 000
Practically insoluble of Insoluble	Greater than or equal to 10,000

7.4. Hygroscopic Nature:

7.4.1. Procedure

2 gm of the test specimens were weighed accurately in petridish and the weight were noted down. Then the test specimens were exposed to 75% RH at 40°C in environment stability testing camber and the other was kept at room temperature for 7 days period. The specimen was weighed after 7 days and the difference in weight was noted down (Table 22).

7.5. Identification of Drug Sample

7.5.1. Finding the absorption maxima (λ max)

The absorption maxima were found for drug identification. Ultraviolet visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the visible/ultraviolet region of the spectrum absorb light of particular wavelength on the type of electronic transition associated with the absorption.

7.5.2. Preparation of Phosphate Buffer Solution [pH 7.4] I.P 1996

- 27.218 g of potassium dihydrogen ortho phosphate was dissolved in 1000 ml of distilled water to give a 0.2N solution
- 8 g of sodium hydroxide was dissolved in 1000ml of distilled water to give 0.2N solution
- 1250ml of 0.2N potassium dihydrogen ortho phosphate and 977.5ml of 0.2N sodium hydroxide were mixed together and made upto 5000ml with distilled water

The drug solution (10, 20, 30, 40, 50, 60 μg/ml) in Phosphate buffer pH 7.4 was taken in standard cuvette, and scanned in the range of 200-300nm in a UV spectrophotometer. It exhibits maxima at 377nm. UV spectrum of drug taken in phosphate buffer pH 7.4 also exhibits maxima at 377nm. Therefore, further all measurements were taken at 377nm. The results are shown in fig 18.

7.6. Standard curve

7.6.1. Preparation of Standard plot for Lornoxicam in phosphate Buffer

pH 7.4

Accurately weighed amount of Lornoxicam (10 mg) was dissolved in small quantity of 0.1N sodium hydroxide and then diluted to 100 ml with phosphate buffer pH 7.4. Each ml of the stock solution contains 100μg of Lornoxicam. From this stock solution different standard of working standard solutions i.e., 10, 20, 30, 40, 50, 60 μg/ml were made up with phosphate buffer pH 7.4 and the absorbance was measured at 377 nm using phosphate buffer pH 7.4 as blank by UV spectrophotometric method. A graph is plotted by using concentration at X-axis and absorbance at Y-axis. The values are given in the table: 25 and fig 19.

7.7. Fourier transforms infrared (FTIR) spectral analysis

FTIR is used to identify the functional groups in the molecule. The drug is mixed with KBr disk was scanned at 4mm/s at a resolution of 2cm over a wave number region of 400 to 4000cm^{-1}. The characteristic peaks were recorded. The results are shown in fig 20, 21, 22, 23, 24, 25 and table 26, 27, 28, 29, 30.

7.8. Drug-Excipient Compatibility Studies by FT-IR Analysis

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. The IR absorption spectra of the pure drug and physical admixtures of drug with various excipients were taken in the

range of 4000-400 cm^{-1} using KBr disc method (Schimadzu IR- Prestige-21) and observed for characteristic peaks of drug.

Drug-Excipient compatibility was carried out by FT-IR analysis. Initially the IR spectrums of pure drug, Lornoxicam, Oleic acid, tween-20, propylene glycol were obtained. After that admixtures of drug with other excipients were prepared and IR Spectra was obtained. The obtained spectra of physical admixtures was observed for major peaks and recorded. The results of this observation were concluded that there is no interaction between the drug (Lornoxicam) and other excipients (Oleic acid, tween-20, propylene glycol).

8. FORMULATION DEVELOPMENT

The pharmaceutical development studies have to be carried out with the purpose of selecting right dosage form and a stable formulation. These studies give detailed description of all the steps involved in the process of development of the finished procedure. Such details are intended towards identifying critical parameters involved in the process, which have to be controlled in order to give reliable and reproducible quality product.

8.1. Dose calculation[78]

The total dose of drug, D_t in a prolonged action preparation comprises the normal (prompt) dose, D_n and the sustaining dose Ds i.e., $D_t = D_n + Ds$ if the first order elimination rate constant is K, the rate at which drug is eliminated when a normal dose is given is $D_n K$ which is the rate at which drug must be replaced if the peak blood level is to be maintained. Given a maintenance period't' the maintenance dose (D_s) is $D_n k_t$. The total dose is therefore:

$$\mathbf{D_t = D_n + D_s}$$

$$= D_n + D_nK_t$$

$$= D_n (1+K_t)$$

$$= D_n (1+0.693t/t1/2)$$

$$\mathbf{D_t = D_i (1+ 0.693 \times t_m/t_{1/2})}$$

Where, D_t = Total dose

D_i = initial dose

t_m = time to which the drug is sustained

$t_{1/2}$ = half life of the drug.

D_i= 10 mg

$t_{1/2}$ = 5 hrs

t_m = 24 hrs

$D_t = 10 (1 + 0.693 \times 24/5)$

D_t= 35.26mg

D_t = 35 mg (app)

8.2. Calculation of HLB value for O/W type of Microemulsions[79]

The HLB of a non-o-ionic surfactant whose only hydrophilic portion is polyoxyethylene is calculated by using the formula

HLB= *E*/5

Where, *E* is the percentage by weight of ethylene oxide. A number of polyhydric alcohol fatty acid esters, such as glyceryl monostearate, can be estimated the formula

HLB= 20(1-*S*/A)

Where, *S* is the saponification number of the ester and A is the acid number of the fatty acid. The HLB of polyoxyethylene sorbitan monolaurate (tween-20),

For which *S*=45.5 and A=276, is

HLB= 20(1-45.5/276) =16.7

The HLB values of some commonly used amphiphilic agents are given in table (13)

The oil phase of an oil-in water (O/W) emulsion requires a specific HLB, called Required Hydrophile- Liphophile Balance (RHLB).A different RHLB is required to form water-in oil (W/O) emulsion from the same oil phase.The RHLB values for both O/W and W/O emulsions have been determined empirically for a number of oil and oil-like substances, some of which are listed in table (14).

Table 13: HLB Values of Some Amphiphilic Agents

Substance	HLB Value
Oleic acid	1
Span-80	4.3
Span-20	8.6
Brij-30	9.5
Tween-80	15
Tween-20	16.7
Sodium oleate	18

Table 14: RHLB for some oil phase ingredients for (O/W) and (W/O) emulsions

Oil phase ingredients	O/W emulsion	W/O emulsion
Cottonseed oil	6-7	-
Mineral oil	10-12	5-6
Castor oil	14	-
Lauric acid	16	-
Oleic acid	17	-

8. 3. Selection of Oils

To find out the suitable oil, which can be used as oil phase in microemulsion, and provide excellent skin permeation rate of lornoxicam. The solubility of lornoxicam in various oils including olive oil, castor oil, isopropyl myristate, isopropyl palmitate, oleic acid was measured at 25°C. The solubility of olive oil, castor oil, isopropyl myristate, isopropyl palmitate, and oleic acid in oily mixtures was also measured[48].

8.3. 1. Procedure:

About 10 gm of oil was accurately weighed in 25 ml glass beaker and 100 mg of lornoxicam was added into it, followed by stirring on magnetic stirrer at moderate speed to dissolve the drug. When drug was dissolved completely another 10 mg lornoxicam of was added and stirring was continued. Addition of drug was continued until the saturated solution is obtained. Finally, the total amount of drug consumed was determined by using UV-spectrophotometer at 377 nm. It was found that, oleic acid has consumed maximum amount of lornoxicam and thus chosen as a vehicle for microemulsion oil phase (15).

Table 15: solubility of lornoxicam in various oils at 25^{o} C

S.no.	Drug solubility (in mg/10 g of oil)	Oils
1	120	Olive oil
2	150	Castor oil
3	140	Isopropyl myristate
4	120	Isopropyl palmitate
5	180	Oleic acid

8.4. Selection of surfactants and co-surfactants[48]

The non-ionic surfactants do not ionize at any great extent in the solution, they are greatly compatible with both anionic and cationic substances; various nonionic surfactants like, span 20, Tween-20 and co-surfactants like, propylene glycol, isopropyl alcohol and b-butanol were subjected to titration. Finally, Tween-20 and propylene glycol were selected as an ideal surfactant and co-surfactant for the system (Table 16).

Table 16: Selection of surfactant and co-surfactants for optimization of formulations

Surfactant: co-surfactant	Concentration ratio	Appearance
Tween-20: propylene glycol	1:1	Clear
	2:1	Clear
Tween-20: isopropyl alcohol	1:1	Slightly cloudy
	2:1	Clear
Tween-20: n-butanol	1:1	Cloudy
	2:1	Clear
Span-20:propylene glycol	1:1	Clear
	2:1	Cloudy
Span-20:isopropyl alcohol	1:1	Slight cloudy
	2:1	Cloudy
Span 20: n-butanol	1:1	Cloudy
	2:1	Cloudy

8.5. Preparation of Lornoxicam Microemulsions by Water Titration Method[39]

O/w type of Microemulsion

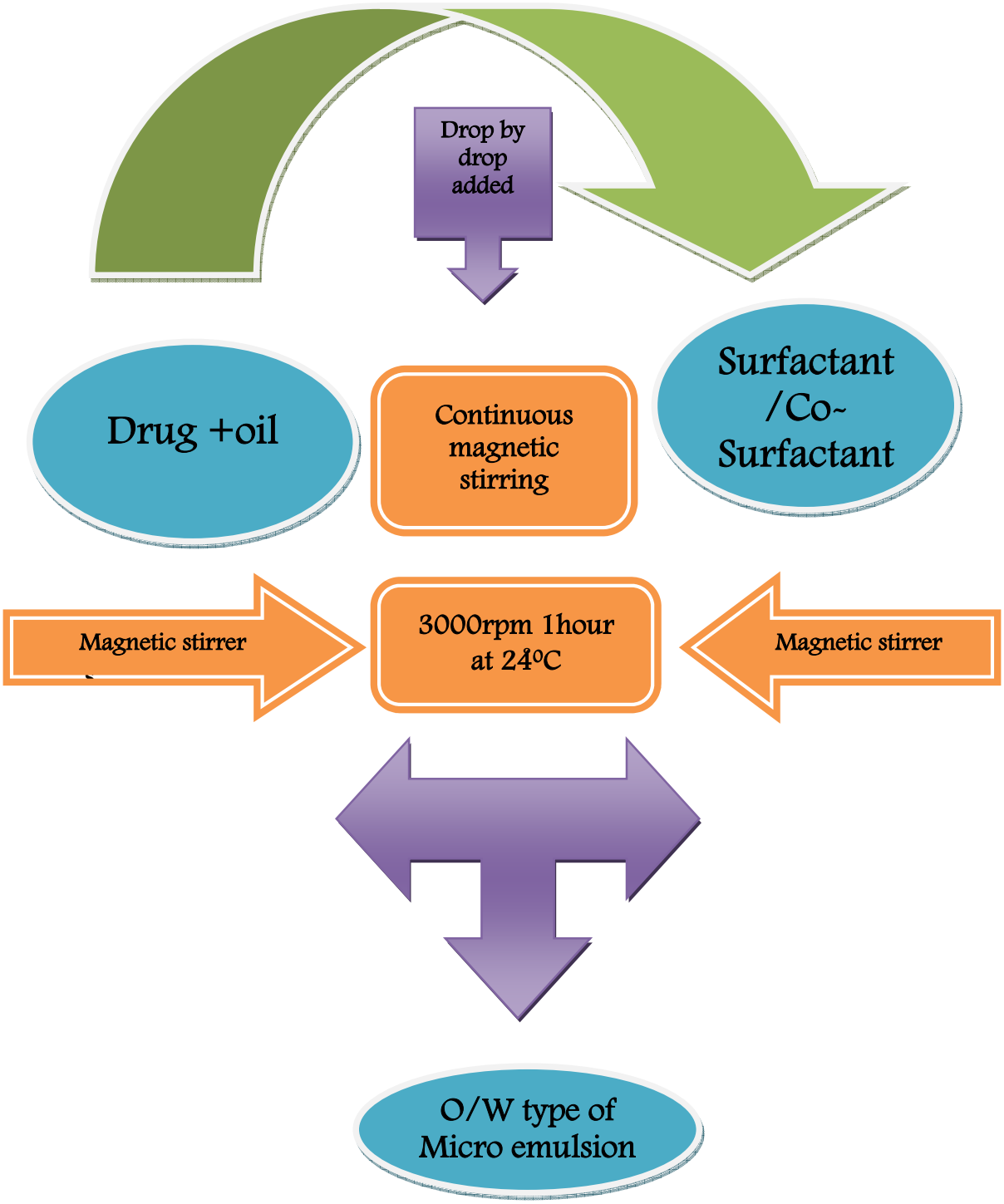


Fig 17: Preparation of Lornoxicam Microemulsions

8.6. Consideration for Formulation Development

- ☞ Preparation of lornoxicam microemulsion trial formulation I and II by optimizing surfactant and co-surfactant ratios (1:1, 2:1)
- ☞ Optimization of microemulsion formulation (Lornoxicam) by optimizing surfactant and co-surfactant is kept constant and oil amount was changed

8.6.1. Trial Formulations

Different trial formulation were formulated and studied for their physicochemical characterization and visual observation. Finally get the optimized formulation.

The trial formulations of microemulsion were prepared based on following formula. Different percentage of surfactant and co-surfactant have been used in each trial formulation and studied to have controlled effect for period of 24 hours.

Trial Batch-I:

The first trial formulations of lornoxicam microemulsion were prepared by employing drug and oil phase same concentration varying the percentage of surfactant and co-surfactant (Tween-20 and Propylene glycol, 1:1ratio). It was shown in the table (17).

Trial Batch-II:

In the second trial formulation, lornoxicam microemulsions were prepared by employing drug and oil phase same concentration and varying the percentage of surfactant and co-surfactant (Polysorbate-20 and Propylene glycol, 2:1ratio). It formulation was shown in the table (18).

Table 17: Formulation of trial batch I (F1-F5)

Surfactant: co-surfactant (1:1)

S.no	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Lornoxicam (mg)	10	10	10	10	10
2	Oleic acid (%w/v)	2	2	2	2	2
3	Tween-20 (%w/v)	1	2	3	4	5
4	Propylene glycol (%w/v)	1	2	3	4	5
5	Distilled water (%w/v)	26	24	22	20	18
6	Final volume (%w/v)	30	30	30	30	30

Table 18: Formulation of trial batch II (F6-F10)

Surfactant: co-surfactant (2:1)

S.no	Ingredients	Formulations				
		F6	F7	F8	F9	F10
1	Lornoxicam (mg)	10	10	10	10	10
2	Oleic acid (%w/v)	2	2	2	2	2
3	Tween-20 (%w/v)	2	4	6	8	10
4	Propylene glycol (%w/v)	1	2	3	4	5
5	Distilled water (%w/v)	25	22	19	16	13
6	Final volume (%w/v)	30	30	30	30	30

Table 19: Compositions of the Selected Microemulsion Formulation

S.no	Formulations	Lornoxicam (mg)	Oleic acid (%W/V)	Tween-20 (%W/V)	Propylene glycol (%W/V)	Distilled water (%W/V)	Final volume (%w/v)
1	ME-1	10	2	6	3	19	30
2	ME-2	10	4	6	3	17	30
3	ME-3	10	6	6	3	15	30
4	ME-4	10	8	6	3	13	30
5	ME-5	10	10	6	3	11	30

9. CHARACTERIZATION OF MICROEMULSIONS

9.1. Optical Transparency[39]

Optical transparency of the formulation was determined by inspecting the sample in clear and transparent container under the presence of good light against reflection into the eyes, and viewed against black and white illuminated background.

9.2. Determination of pH

pH is measured using a pH meter of a glass electrode. pH fundamentally represents the value of hydrogen ion activity in solutions. It is defined by the equation given below. This value well accords with the logarithm of the reciprocal of hydrogen ion concentration in dilute solutions.

$$pH = pHS + \frac{E - ES}{2.3026\ RT/F}$$

Where, pHs = pH value of a pH standard solution,

E = electromotive force (volt) on the combination of glass and reference Electrodes in a sample solution; the constitution of the cell

Es = electromotive force (volt) on the combination of glass and reference Electrodes in a pH standard solution, the constitution of the cell

R = gas constant,

T = absolute temperature,

F = Faraday constant.

The values of 2.3026 RT/F (volt) at various temperature of solutions.

The pH was measured in microemulsion formulations using a ELICO LI120 pH meter that was calibrated before formulation use with buffered solutions at pH 4 and pH 9.2.

A defined amount of formulation was taken and diluted with calibrated distilled water and mixed well. The electrode of the pH meter was immersed in the prepared formulation for pH determination.

About 2gm of formulation was dispersed into 20ml of distilled water and pH was determined by pH meter.

9.3. Viscosity Measurements

This procedure determines the viscosity of a fluid by the use of a Brookfield Viscometer. Viscosity is the measure of fluid friction which can be considered as the internal friction resulting when a layer of fluid is made to move in relationship to another layer. Viscosity is a measure of the ratio of shearing stress to rate of shear.

$$\frac{\textbf{Shear Stress (dynes)}}{\textbf{Rate of Shear (cm/sec)}} = \textbf{Poise}$$

- Check to confirm that the viscometer has been calibrated. If not, calibrate using software.
- The sample container and quantity should be approximately the same as for the Calibration Standard. Equilibrate the temperature of the sample to the temperature designated in the specification (±1°C).
- Confirm that the viscometer is level using the bubble level on the back of the instrument. For the Brookfield LV-II, the instrument with spindle attached and the speed set as designated in the product specification. The main display will flash 00.0 after 10 seconds.
- Immerse the spindle designated in the product specification into the sample to the groove on the spindle shaft. Do not allow air bubbles to be formed. Attach the spindle to the viscometer.
- The spindle should not touch the bottom or sides of the container and should be centered. Reconfirm that the viscometer in level.

- The spindle no: 64 were rotated at a speed of 60 rpm. Samples of microemulsions were allowed to settle over 30 min at room temperature before the measurements were taken.
- For the LV-II, choose the units by pressing the desired unit key (CPS for centipoises).
- Set the speed as designated in the product specification, start the viscometer and read at constant reading. For manual models, use the conversion chart to convert the dial readings to centipoises.
- When done, turn motor and power off. Clean spindle and place in spindle holder.

9.4. Mechanical stress study[30]

The chemical and physical stability of microemulsion with lornoxicam were evaluated *via* phase separation by mechanical stress study**.**

The different microemulsion formulations (ME-1 to ME-5) were centrifuged (Remi centrifuge) at 2000 rpm for different time interval (10min, 30min, and 60min) and noted down the volume of phase separation of formulation.

9.5. Particle shape and Surface Morphology

9.5.1. Transmission Electron Microscopy (TEM)

Morphology and structure of the microemulsion were studied using transmission electron microscopy with Topcon 002B operating at 200kv (Topcon, Paramus, NJ) and capable of point-to-point resolution. In order to perform transmission electron microscopy observations, a drop of the microemulsion was suitably diluted with water and applied on a carbon-coated grid, then treated with a drop of 2% phosphotungstic acid and left for 30s.The coated grid was dried under vacuum and then taken on a grid holder and observed under the transmission electron microscope**.**

9.5.2. Atomic Force Microscopy (AFM)

An atomic force microscope is an excellent for visualising particles with sizes ranging from 1 nm to 10 µm. Another advantage of the AFM is its simplicity of operation and that the AFM requires minimal sample preparation. Additionally, the AFM can operate in air, liquid or a vacuum. In comparison to traditional techniques for single particle analysis of sub-µm particles, the AFM gives three-dimensional profiles.

It is possible to make quantitative measurements of particle sizes with an AFM. It can easily measure particle sizing parameters as long as the particle is >100 nm. If the particle size is less than 100 nm special considerations must be taken into account.

9.6. Particle Size Measurement

9.6.1. Determination of particle size distribution by Particle size analyzer:

The selected best Lornoxicam microemulsion formulations were subjected to laser particle counting method. Here the sample was injected into the sample delivery and controlling chamber. Then, suitable solvent was pumped through the chamber. Now a beam of laser light was allowed to fall on the sample cell. After required number of runs, they were directed towards the detector. From this the particle size range and the average mean particle size of the formulation can be studied. The average particle size of Microemulsion formulations can be determined using particle size analyzer.

9.7. Drug content analysis[30]

1ml of Microemulsion Formulations was transferred into a beaker containing 10 ml methanol. The content of the beaker were stirred for 30 minutes and then kept for 24hr. After 24hr the content of beaker were transferred into centrifuge tube and centrifuged at the 3000 rpm for 10 min. Supernatant was separated and filtered. Then 0.1 ml of the supernatant was diluted appropriately with Phosphate Buffer Saline (PBS) pH 7.4 and assayed Spectrophotometrically for drug contant.

10. *IN VITRO* SKIN PERMEATION STUDY,

10.1. Preparation of Goat skin[26]:

Selected formulations were further studied for skin permeation using goat ear skin, obtained from the slaughter house after sacrificing the animal within 1 hour. The average thickness of the goat skin was 0.28±0.06 mm and then the hair was removed from the upper portion of skin surface using an animal hair clipper, and, subsequently, full thickness of the skin was harvested. The fatty layer, adhering to the dermis side, was removed by surgical scalpel. Finally, these excised skins were thoroughly rinsed with distilled water and packed in aluminum foils. The skin samples were stored at -20°C and used within a week.

10.1.1. *In Vitro* Skin Permeation Study

In-vitro permeation study of drug from ME-1 to ME-5 lornoxicam microemulsion formulations was carried out using *Goat Skin*. The average thickness of the skin was 0.28±0.06 mm. Skins were allowed to hydrate for 1 hour before being mounted on the open ended diffusion with the stratum corneum facing the donor compartment and the dermal side faced the receiver compartment.

The receptor compartment was consist of 400mL of phosphate buffer (pH 7.4) in 500 mL beaker and its temperature was maintained at 37 ±0.5°C and stirred at 300 rpm throughout the experiment. About 1gm of 1% lornoxicam microemulsion was placed in *Goat Skin* tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer. Samples were taken from receptor mediums at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8, 9, 10, 11, 12, 23, 24hrs and replaced immediately with an equal volume of fresh phosphate buffer equilibrated at 37 ±0.5°C. All the samples were analyzed for lornoxicam content at 377nm by UV-spectrophotometer. Cumulative amount of drug permeation was calculated from standard calibration curve.

Permeation Study:

Apparatus	**:**	Open ended diffusion cylinder
Speed	**:**	300rpm
pH	**:**	7.4
Time	**:**	1-24hrs
Temperature	**:**	37□ C
λmax	**:**	377nm

10.2. Kinetics of drug release[80, 81, 82]

Several theories and kinetic models describe the dissolution of drug from immediate release and modified release dosage forms. There are several models to represent the drug dissolution profiles where f (t) is a function of time related to the amount of drug dissolved from the pharmaceutical dosage form.

The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of a generic equation that mathematically translates the dissolution curve function of some parameters related with the pharmaceutical dosage forms. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time‘t’ or Q(t). Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell models, Weibull models. These models are used to characterize drug dissolution/release profiles.

10.2.1. (i) Zero Order Kinetics

This model represents an ideal release profile in order to achieve the pharmacological prolonged action. Zero order release constitutes drug release from the dosage form that is independent of the amount of drug in the delivery system (that is, a constant release rate). The following equation is used to express the model:

$$Q_t = Q_o + K_o t$$

Where, Q_t is the amount of drug dissolved in time t

Q_o is the initial amount of drug in the solution

K_o is the zero order release constant

For practical purposes the equation is rearranged:

Percent drug released = Kt

This is applicable to dosage forms like transdermal systems, coated dosage forms, osmotic systems as well as matrix tablets with low soluble drugs.

10.2.2. (ii) First Order Kinetics

First order release constitutes drug release in a way that is proportional to the amount of drug remaining in its interior; in such a way that amount of drug released by unit time diminish. The following equation is used to express the model:

$$\mathbf{\log Q_t = \log Q_o + Kt/2.303}$$

Where, Q_t is the amount of drug dissolved in time t

Q_o is the initial amount of drug in the solution

K is the first order release constant

For practical purposes the equation is rearranged:

Log % of drug unreleased = Kt/2.303

This model is applicable to dosage forms such as those containing water-soluble drugs in porous matrices.

10.2.3. (iii) Higuchi Model

Higuchi describes drug release as a diffusion process based in Fick's law,square root dependent. The following equation is used to express the model:

$$\mathbf{Q_t = K_h t^{1/2}}$$

Where, Q_t is the amount of drug dissolved in time t

K_h is the first order release constant

For practical purposes the equation is rearranged:

Percent drug released = $Kt^{1/2}$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drugs.

10.2.4(iv) Peppas-Korsmeyer Model

This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved

The following equation is used to express the model

$$\mathbf{Q_t/Q_\infty = Kt^n}$$

Where, Q_t is the amount of drug dissolved in time t

Q_∞ is the amount of drug dissolved in infinite time

n is the release exponent indicative of drug release mechanism

K is the kinetic constant

For practical purposes the equation is rearranged:

Log percent drug released = log k +n log t

Peppas used n value in order to characterize different release mechanism concluding for values of n = 0.5 for Fickian diffusion and values of n, between 0.5 to 1.0 for anomalous transport (corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics) and higher values of n, n=1 or n>1 for case-II transport (corresponds to erosion and relaxation of swollen polymer layer).

10.3. *In-vivo* study design[63]

10.3.1. Anti-inflammatory activity:

Materials and methods:

Chemicals:

- Carrageenan, type I, Sigma-Aldrich Louis, MO, (USA);
- Lornoxicam microemulsion(ME-3) formulation as prepared in our lab. at department of Pharmaceutics.

The anti-inflammatory and sustaining action of the optimized formulation (ME-3) was evaluated by the carrageenan-induced hind paw edema method developed by Winter et al in Wistar rats.

Animals:

White male albino rats weighting between (170gm and 200 gm) were selected for evaluation of the anti-inflammatory activity by measurement of oedema size resulting from carrageenan injection in the right hind paw region of the body. The animals were kept under standard laboratory conditions with free access to a standard laboratory diet and water ad libitum. The dose for the rats was calculated based on the weight of the rats according to the surface area ratio.

Treatment:

The animals were divided into two groups, consisting of (six animals per each).

Group 1:- Control group treated with non medicated microemulsion.

Group 2:- Treated group with Lornoxicam- microemulsion

Paw oedema size induced by carrageenan injection:

Certain amount of the investigated microemulsion (100 mg) was applied topically to the right hind paw of the rats. The area of application is lightly occluded with bandages and it was left in place for two hours. The dressing was then removed and the microemulsion remaining on the surface of the skin was wiped off with a piece of cotton.

The paw volume was determined immediately before carrageenan injection and considered as zero time. The animals were then injected with 0.1 ml of 1% freshly prepared sterile carrageenan solution in saline into sub-plantar region of right hind paw of rats.

The contralateral paw received an equal volume of saline. The right hind paw thickness was measured from ventral to dorsal surfaces, with a dial caliper, after 0.5, 1, 2, and 3 hrs after the sub-plantar injection of carrageenan.

The size of oedema which expressed as a percentage change in paw thickness (in mm) from control (pre-drug, zero time) and measured by digital plethysmometer fig: 59. The experimental setup has shown in Fig 60. The amount of paw swelling was determined for 3 hours and expressed as percent oedema relative to the initial hind paw volume. The percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group.

10.4. Statistical analysis:

It was carried out by Student' t-test using Excel by Graph pad software, to determine the significance of the obtained results between the prepared lornoxicam microemulsion.

The % of the effect (inhibition) was calculated by the following

Equation: = [(Control – drug)/ control] x 100.

11. STABILITY STUDY

Nowadays, stability testing has become an integral part of formulation development. It generates information on which proposal the shelf life of drug or dosage form and their recommended storage conditions are based.

11.1. Accelerated stability studies

Definition:

Stability of a pharmaceutical preparation can be defined as the capability of a particular formulation (dosage form or drug product) in a specific container /closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf- life.

Purpose of stability testing:

- To study of drug decomposition kinetics
- To develop stable dosage form
- To establish the shelf- life or expiration date for commercially available drug product
- To ensure the efficacy, safety and quality of active drug substances and dosage forms

ICH Guidelines – Specifications[83]

- 5% potency loss from initial assay of batch
- Any specified degradation that exceed specifications
- Product failing out of pharmacopoeial limits.
- Dissolution out of specification for 12 minutes.
- Failure to meet specification for appearance and physical properties.

Any one condition is observed then stability of the batch is failed.

Table 20: Stability Storage Conditions[84, 85]

S.No.	Study Period	Storage Condition	Minimum Duration
1	Longer	25 ± 2° C 60 ± 5% RH	6 Months
2	Intermediate	30 ± 2 ° C 65 ± 5% RH	3 Months
3	Accelerated	40 ± 2° C 75 ± 5% RH	3 Months

Stability of microemulsion formulations on storage is of a great concern as it is the major problem in the development of marketed preparation. Selected microemulsion formulations (ME-3) were placed in a high density polyethylene container and kept in stability chamber maintained at 40°c and 75% RH. The stability studies were carried out for a period of three months. The microemulsion formulation were tested and checked at regular intervals for changes in percentage of drug content. The results are discussed in table20.

12. RESULTS AND DISCUSSION

12.1. Preformulation studies

Description:

Nature : Yellow crystalline powder

Taste : weakly acidic drug

Melting point:

Table 21: Melting Point Determination

Drug	*Melting point (°)	Normal range (°)
Lornoxicam	240 ± 0.145	239-241

* All values are expressed as Mean ± S.D, n=3.

Solubility:

The solubility of drug in various solvents was shown in the table

Table 22: Solubility Profile of Lornoxicam

S. No	Solvent	Solubility
1.	Distilled water	Slightly Soluble
2.	Phosphate buffer (pH 7.4)	Very Soluble
3.	Methanol	Very Soluble
4.	Ethanol	Slightly Soluble
5.	Chloroform	Slightly soluble
6.	0.1N NaoH	Very soluble

Hygroscopic Nature:

Table 23: Hygroscopic Nature of LX

At Room Temperature	75% RH at 40°
Sample No-1	Sample No-1
Weight Gain Observed-Nil	Weight Gain Observed-Nil

Lornoxicam is non hygroscopic in Nature

12.2. Identification of Drug Sample

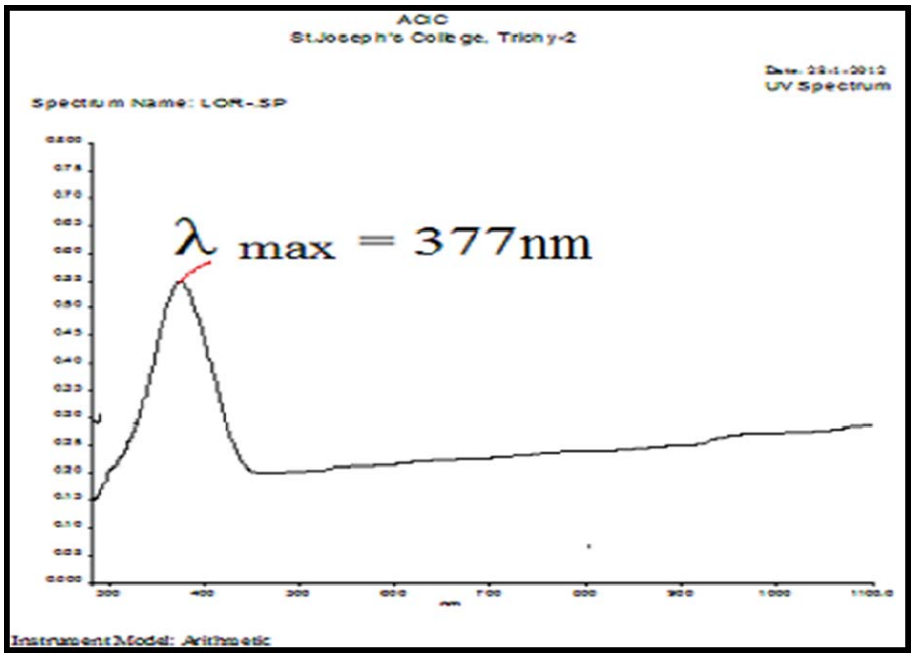


Fig 18: UV spectrum of Lornoxicam in phosphate buffer pH 7.4

Table 24: Absorption maxima of Lornoxicam in phosphate buffer pH 7.4

Solvent	Concentration (μg)/ml	λ max (nm)	Absorbance
Phosphate buffer pH 7.4	60	377	0.6702

Standard plot of Lornoxicam in phosphate buffer pH 7.4

Table 25: UV Absorbance of phosphate buffer pH 7.4

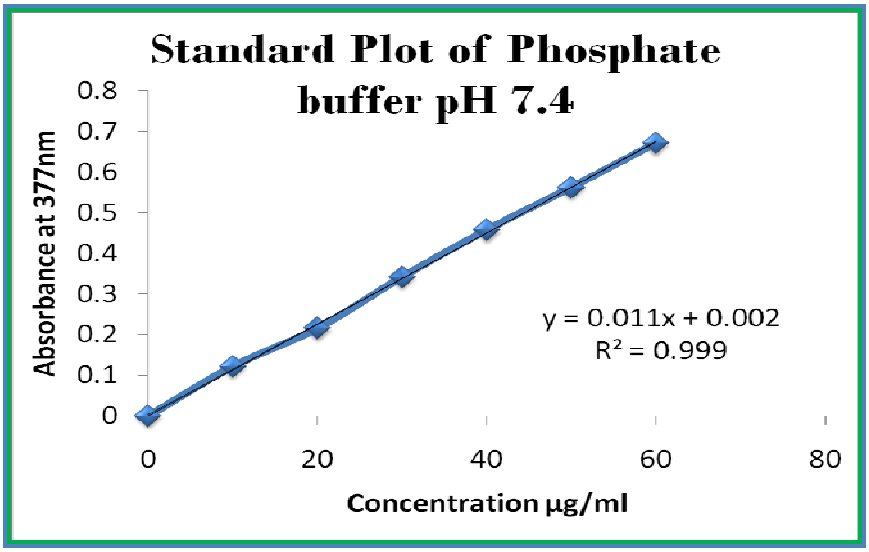
S.No.	Concentration (μg/ml)	Absorbance at 377nm
1	10	0.1219
2	20	0.2151
3	30	0.3413
4	40	0.4568
5	50	0.5615
6	60	0.6702

sa = 0.002

b = 0.011

r = 0.999

Fig 19: Standard plot of Lornoxicam in pH 7.4



The Standard plot has good regression coefficient and it shows the linearity.

12.3. FTIR

Fig 20: FT-IR of Lornoxicam

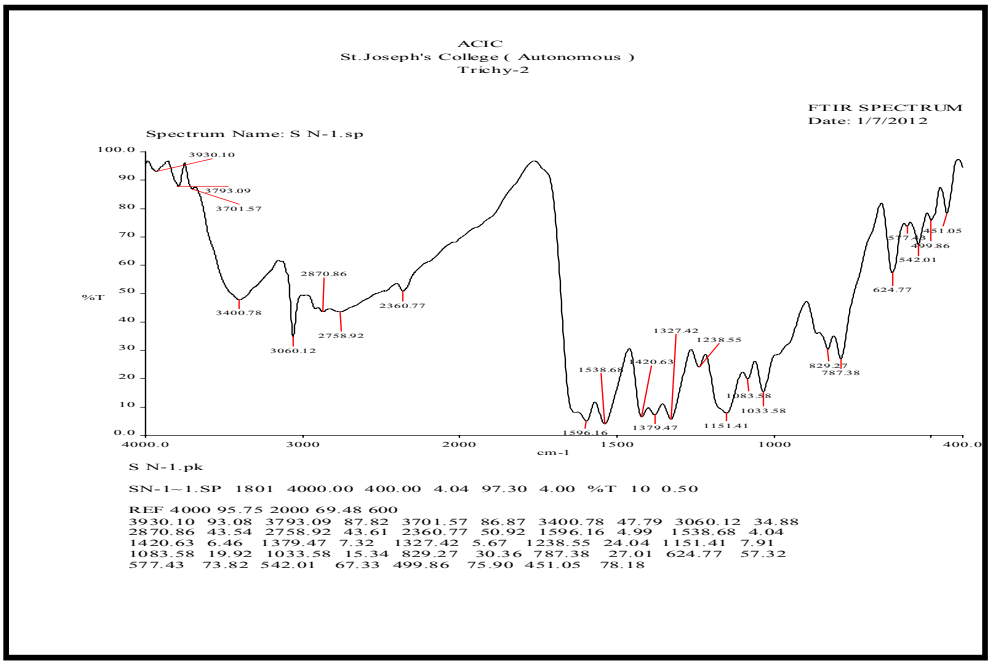


Table 26: FT-IR Spectral assignment of Lornoxicam

Wavenumber in (cm^{-1})	Functional groups
3400.78	O-H stretching
3060.12	N-H stretching
2870.86,2758.92	C-H(Aromatic) stretching
1596.16	Carbonyl –C=O stretching
1538.68	NH(Amide) stretching
1420.63	S=O stretching
1379.47	C-S Stretching
1238.55	C-O Stretching
1151.41	C-N Stretching
829.27,624.77,451.05	C-H outplane bending

Fig 21: FT- IR of oleic acid

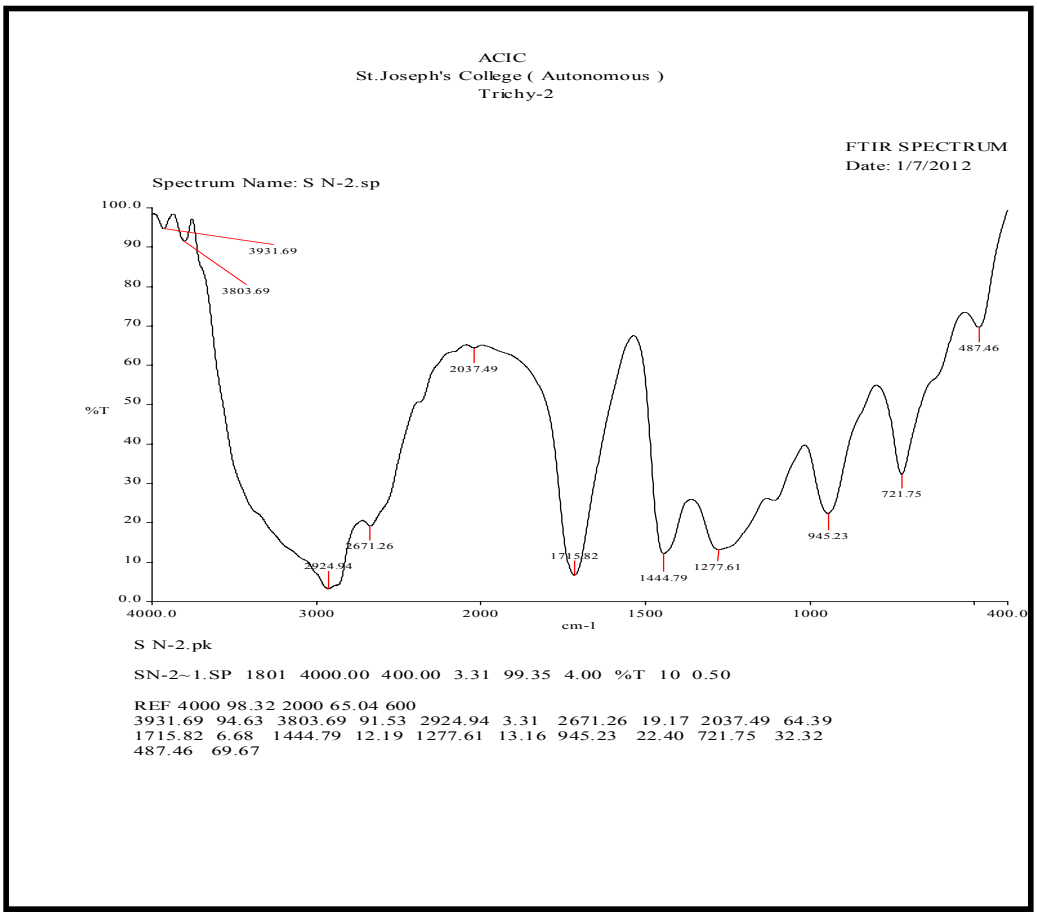


Table 27: FT-IR spectral assignment of Oleic acid

Wavenumber in (cm^{-1})	Funtional groups
2924.94	C-H Stretching
1715.82	C-O Stretching
1277.61	C-O Stretching
721.75	C-H Out of plane bending

Fig 22: FT-IR of Tween-20

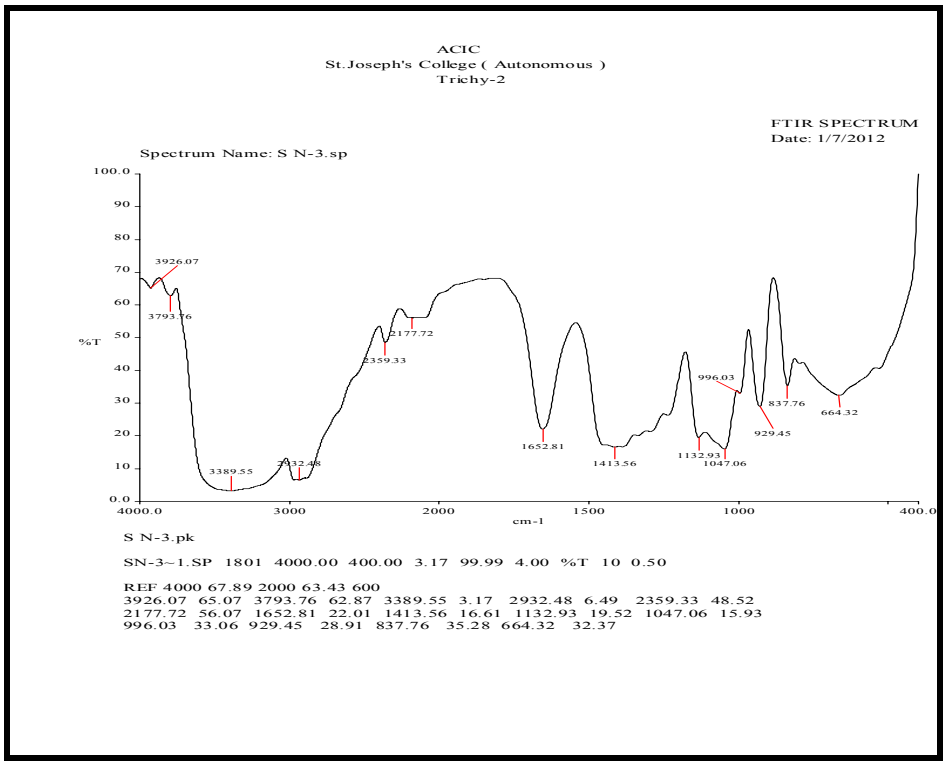


Table 28: FT-IR spectral assignment of Tween-20

Wavenumber in (cm^{-1})	Funtional groups
3389.55	N-H Stretching in Primary amine
2932.48	C-H Stretching
837.76	C-O Stretching
664.32	C-H Out of plane bending

Fig 23: FT-IR of Propylene glycol

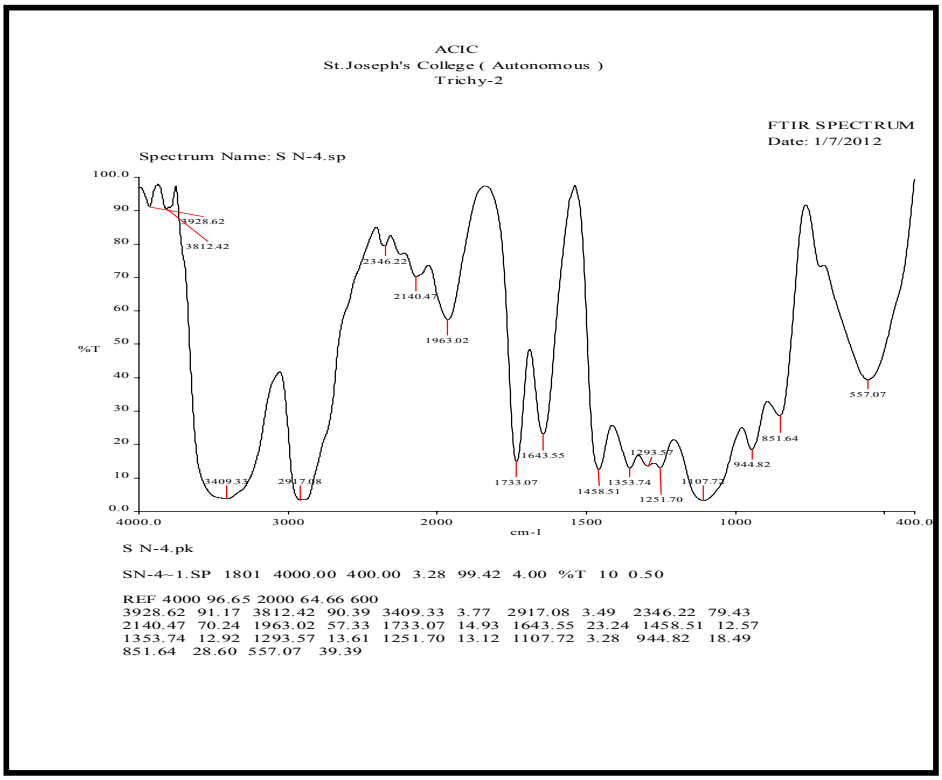


Table 29: FT-IR spectral assignment of Propylene glycol

Wavenumber in (cm^{-1})	Funtional groups
3409.62	O-H Stretching
2917.08	C-H Stretching
1251.70	C-O Stretching

Fig 24: FT-IR of physical Admixture 1. (Lornoxicam + Oleic acid + Tween-20 + Propylene glycol)

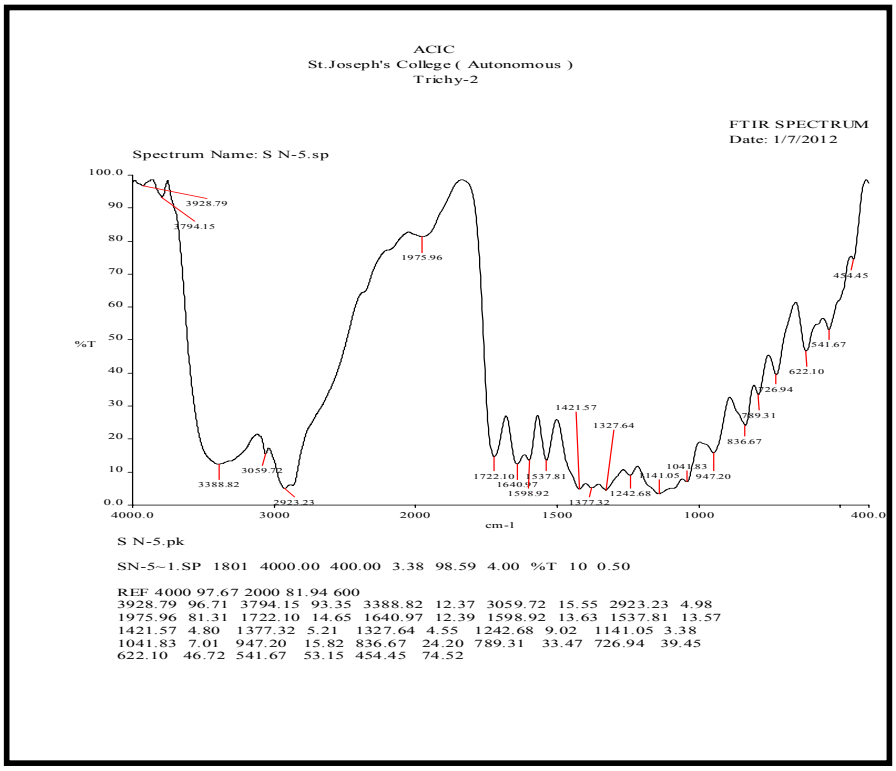


Table 30: FT-IR spectral assignments Physical admixtures-1, Lornoxicam + Oleic acid + Tween-20 + Propylene glycol

Wavenumber in (cm^{-1})	Assignment
3388.82	O-H Stretching
3059.72, 2923.23	C-H Stretching
1722.10	C=O Stretching
1598.92	C-N Stretching
1327.64	N=O Stretching

There are no extra peaks seen other than the normal peak in the spectra of the mixture of the drug and excipients and so there is no interaction with the drug and excipient and they are compatible with each other.

The IR spectra of the drug and polymer combination were compared with the spectra of the pure drug and individual excipients in which no shifting of peaks was significantly found, indicating the stability of the drug during microemulsion formulation development.

12.2. Characterization of Microemulsions

12.2.1. Optical transparency

Table 31: Appearance of Formulations

Formulation	Appearance
ME-1	Milky
ME-2	Opalescent
ME-3	Clear
ME-4	Milky
ME-5	Milky

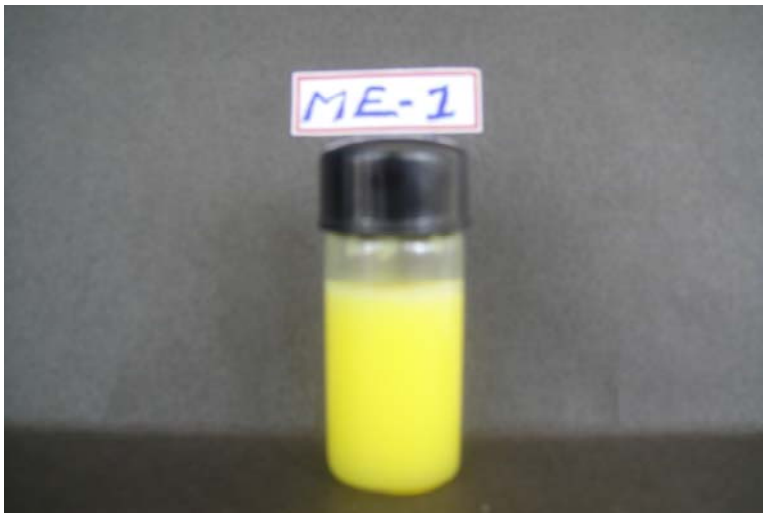


Fig 25: ME-1

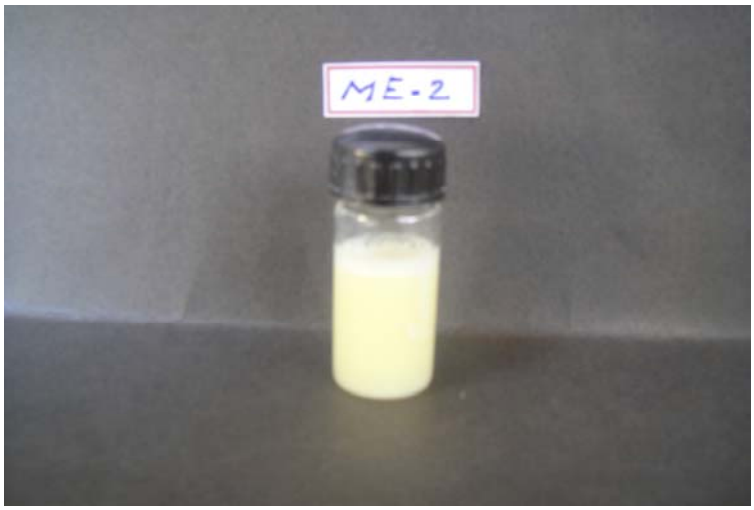
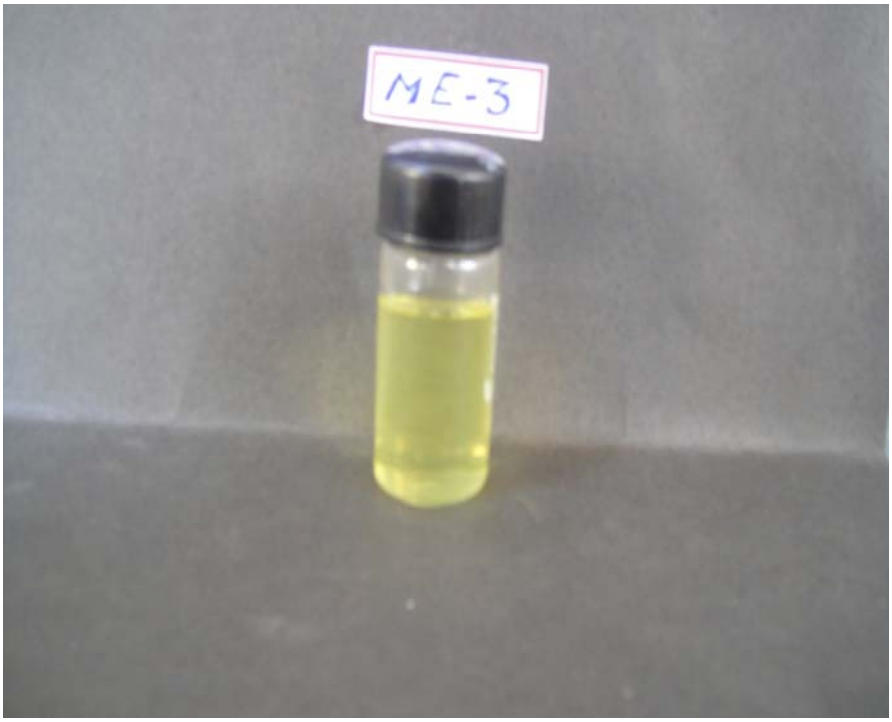


Fig 26: ME-2

Fig 27: ME-3



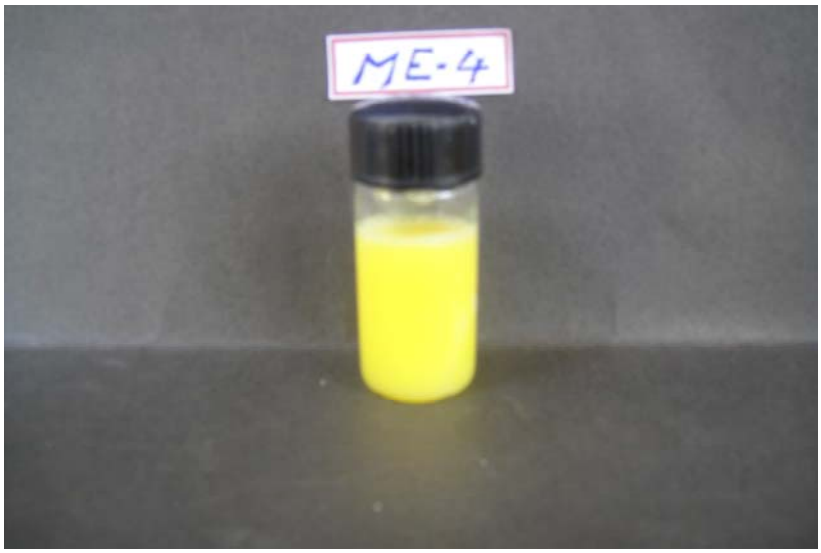


Fig 28: ME-4

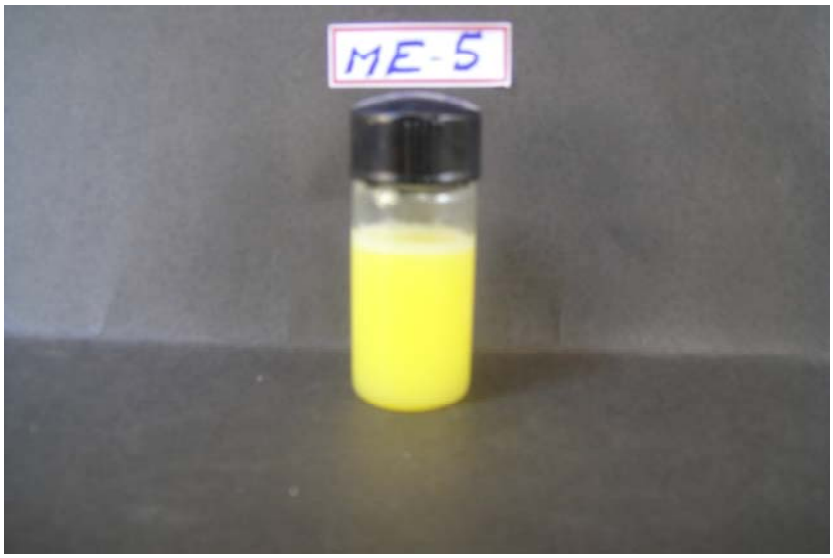


Fig 29: ME-5

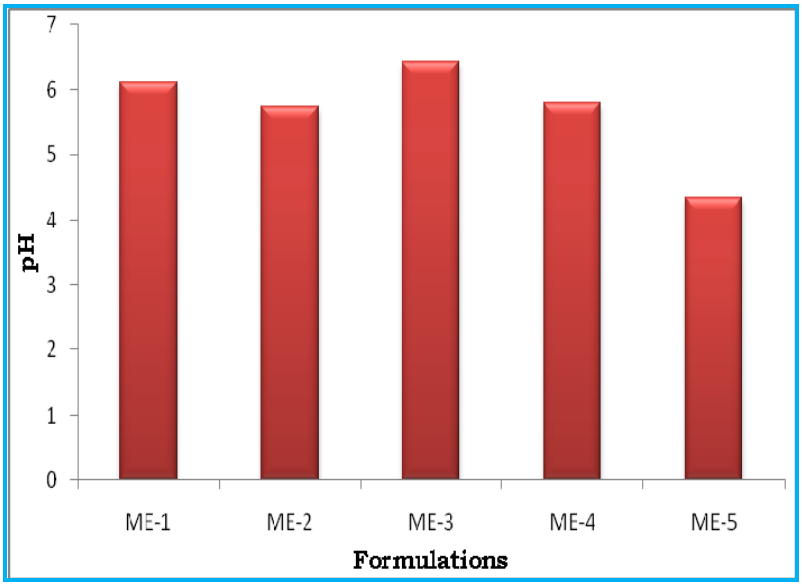
pH determination

Table 32: Comparative pH values of Formulations

Formulations	pH*
MEI	6.12±0.04
ME2	5.75±0.03
ME3	6.42±0.02
ME4	5.81±0.03
ME5	4.35±0.06

***Values are mean** ±SD, n=3

Fig 30: Comparative pH values of Formulations



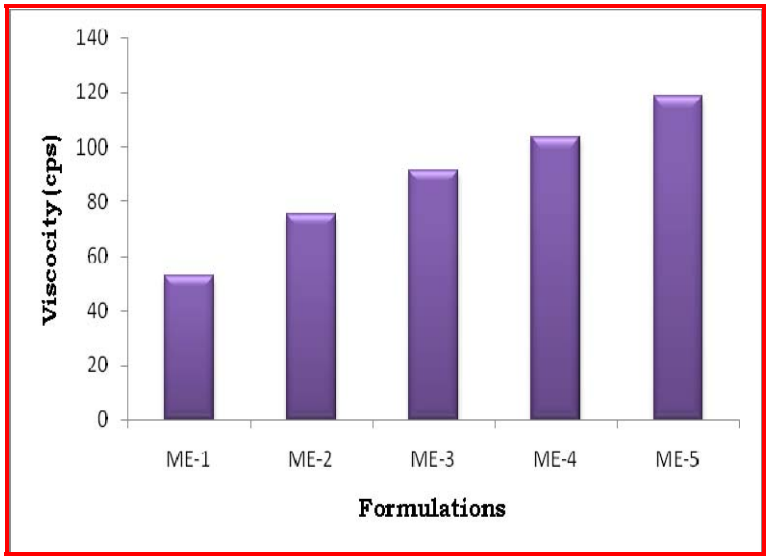
Viscosity measurements

Table 33: Comparative Viscosity values of Formulations

Formulations	Viscosity(cps)*
ME-1	52.6±0.6
ME-2	75.3±0.8
ME-3	91.4±0.4
ME-4	103.5±0.5
ME-5	118.2±0.2

***Values are mean** ±SD, n=3

Fig 31: Comparative Viscosity values of Formulations

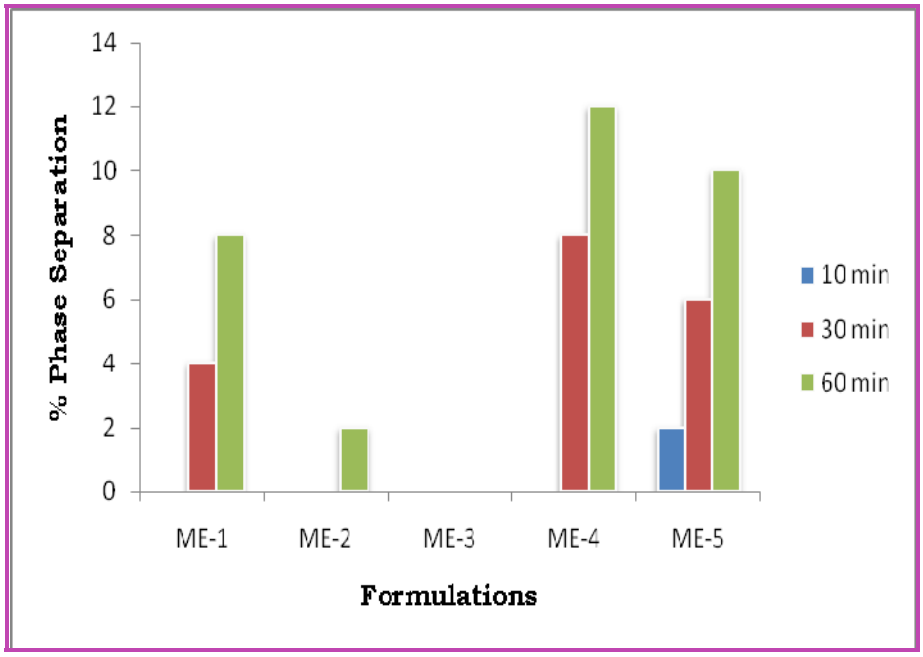


Mechanical stress study

Table 34: Comparative studty of mechanical stress in Formulations

S.No	Centrifugation time (min)	% Phase separation				
		ME-1	ME-2	ME-3	ME-4	ME-5
1	10	-	-	-	-	2
2	30	4	-	-	8	6
3	60	8	2	-	12	10

Fig 32: Comparative studty of mechanical stress in Formulations



TEM PHOTOGRAPH OF FORMULATION (ME-3)

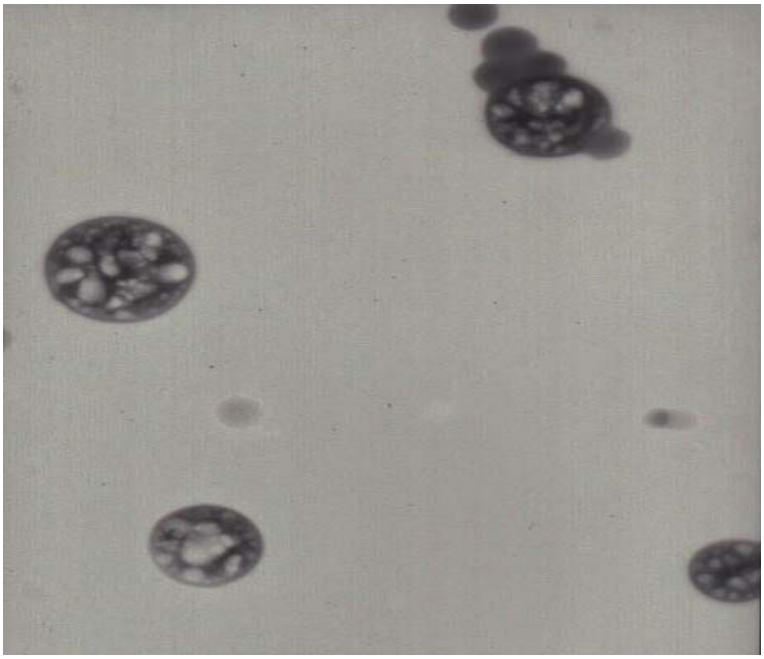


Fig: 33

AFM PHOTOGRAPH OF FORMULATION (ME-3)

Fig (a) 34: particle size range of Formulation (ME-3)

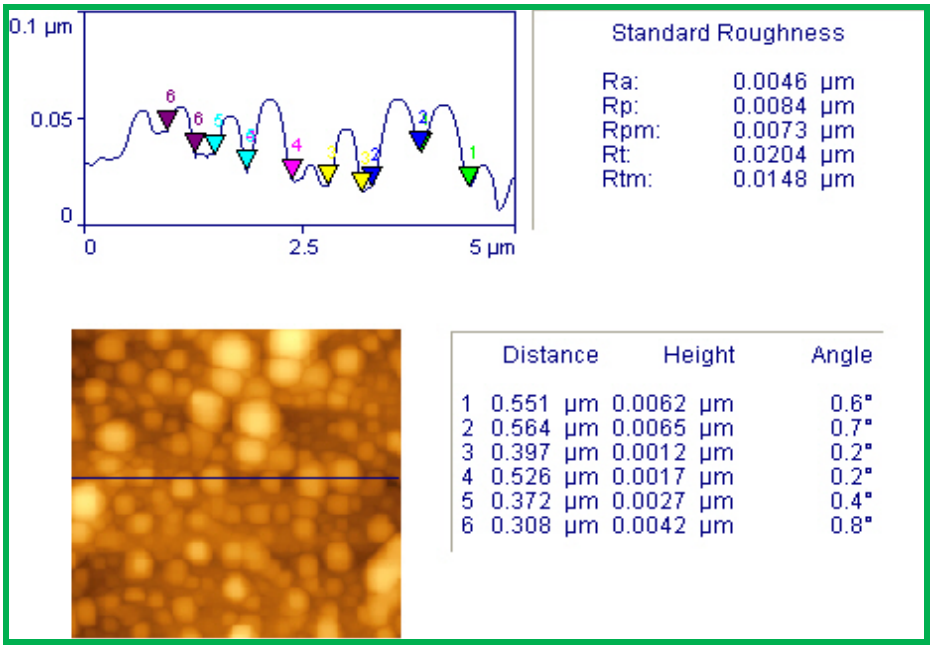
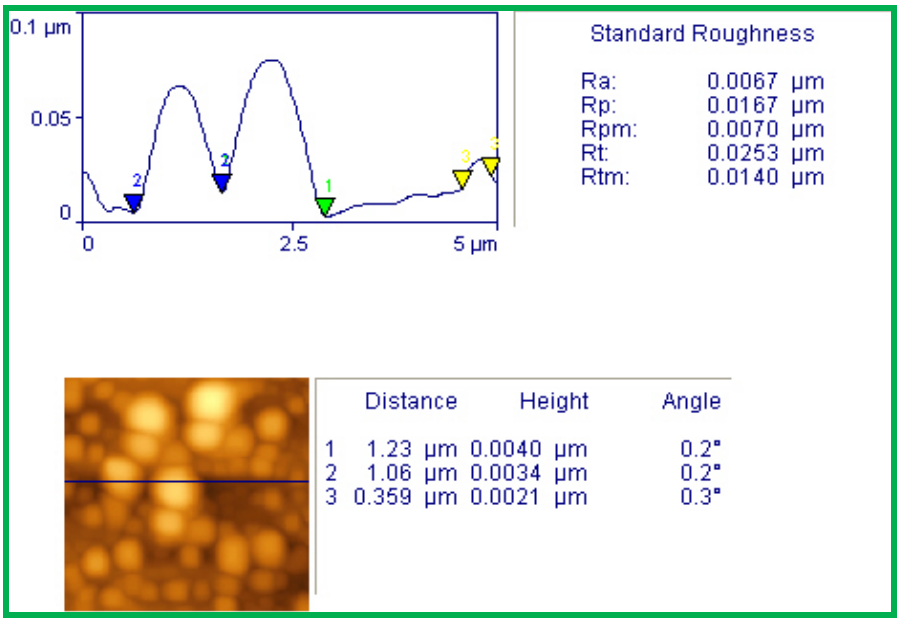
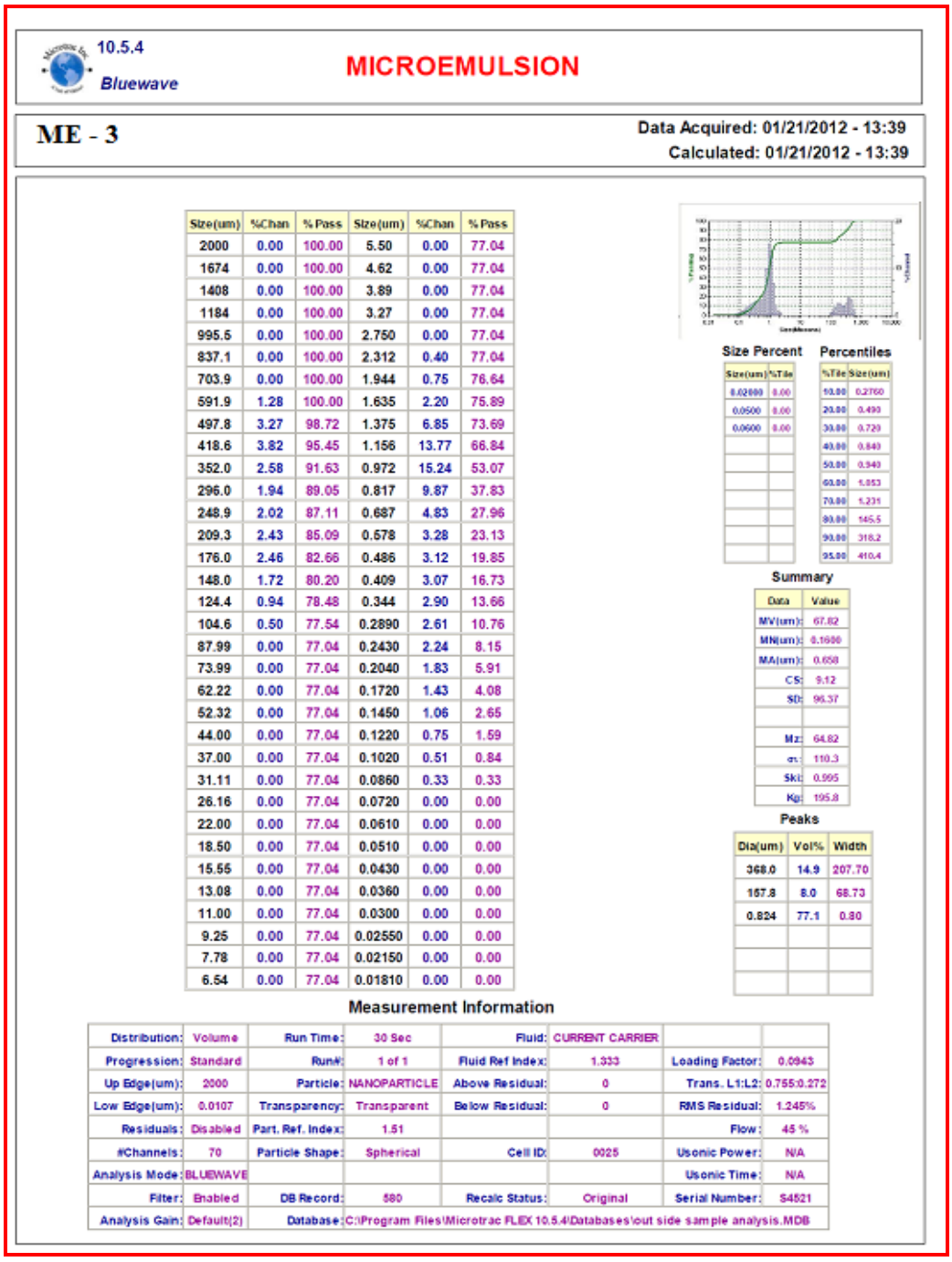


Fig (b) 35: particle size range of (ME-3)



Determination of particle size



10.5.4 Bluewave

MICROEMULSION

ME - 3

Data Acquired: 01/21/2012 - 13:39
Calculated: 01/21/2012 - 13:39

Size(um)	%Chan	% Pass	Size(um)	%Chan	% Pass
2000	0.00	100.00	5.50	0.00	77.04
1674	0.00	100.00	4.62	0.00	77.04
1408	0.00	100.00	3.89	0.00	77.04
1184	0.00	100.00	3.27	0.00	77.04
995.5	0.00	100.00	2.750	0.00	77.04
837.1	0.00	100.00	2.312	0.40	77.04
703.9	0.00	100.00	1.944	0.75	76.64
591.9	1.28	100.00	1.635	2.20	75.89
497.8	3.27	98.72	1.375	6.85	73.69
418.6	3.82	95.45	1.156	13.77	66.84
352.0	2.58	91.63	0.972	15.24	53.07
296.0	1.94	89.05	0.817	9.87	37.83
248.9	2.02	87.11	0.687	4.83	27.96
209.3	2.43	85.09	0.578	3.28	23.13
176.0	2.46	82.66	0.486	3.12	19.85
148.0	1.72	80.20	0.409	3.07	16.73
124.4	0.94	78.48	0.344	2.90	13.66
104.6	0.50	77.54	0.2890	2.61	10.76
87.99	0.00	77.04	0.2430	2.24	8.15
73.99	0.00	77.04	0.2040	1.83	5.91
62.22	0.00	77.04	0.1720	1.43	4.08
52.32	0.00	77.04	0.1450	1.06	2.65
44.00	0.00	77.04	0.1220	0.75	1.59
37.00	0.00	77.04	0.1020	0.51	0.84
31.11	0.00	77.04	0.0860	0.33	0.33
26.16	0.00	77.04	0.0720	0.00	0.00
22.00	0.00	77.04	0.0610	0.00	0.00
18.50	0.00	77.04	0.0510	0.00	0.00
15.55	0.00	77.04	0.0430	0.00	0.00
13.08	0.00	77.04	0.0360	0.00	0.00
11.00	0.00	77.04	0.0300	0.00	0.00
9.25	0.00	77.04	0.02550	0.00	0.00
7.78	0.00	77.04	0.02150	0.00	0.00
6.54	0.00	77.04	0.01810	0.00	0.00

Size Percent

Size(um)	%Tile
0.02880	0.00
0.0500	0.00
0.0600	0.00

Percentiles

%Tile	Size(um)
10.00	0.2760
20.00	0.493
30.00	0.720
40.00	0.940
50.00	0.940
60.00	1.053
70.00	1.231
80.00	145.5
90.00	318.2
95.00	410.4

Summary

Data	Value
MV(um):	67.82
MN(um):	0.1686
MA(um):	0.658
CS:	9.12
SD:	96.37
Mz:	64.82
σι:	110.3
Ski:	0.995
Kg:	195.8

Peaks

Dia(um)	Vol%	Width
368.0	14.9	207.70
157.8	8.0	68.73
0.824	77.1	0.80

Measurement Information

Distribution:	Volume	Run Time:	30 Sec	Fluid:	CURRENT CARRIER		
Progression:	Standard	Run#:	1 of 1	Fluid Ref Index:	1.333	Loading Factor:	0.0943
Up Edge(um):	2000	Particle:	NANOPARTICLE	Above Residual:	0	Trans. L1:L2:	0.755:0.272
Low Edge(um):	0.0107	Transparency:	Transparent	Below Residual:	0	RMS Residual:	1.245%
Residuals:	Disabled	Part. Ref. Index:	1.51			Flow:	45 %
#Channels:	70	Particle Shape:	Spherical	Cell ID:	0025	Usonic Power:	N/A
Analysis Mode:	BLUEWAVE					Usonic Time:	N/A
Filter:	Enabled	DB Record:	580	Recalc Status:	Original	Serial Number:	S4521
Analysis Gain:	Default(2)	Database:	C:\Program Files\Microtrac FLEX 10.5.4\Databases\out side sample analysis.MDB				

Fig 36: Particle size Measurement of ME-3

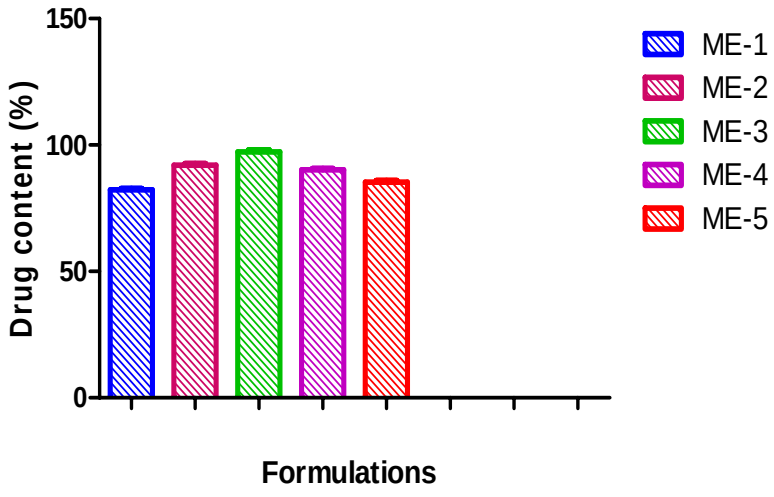
Drug content:

Table 35: Comparative drug content of Formulations

Formulations	Drug content (%)*
ME-1	82.42±0.32
ME-2	93.12±0.54
ME-3	98.54±0.26
ME-4	90.21±0.42
ME-5	86.34±0.28

***Values are mean** ±SD, n=3

Fig 37: Comparative drug content of Microemulsion formulations



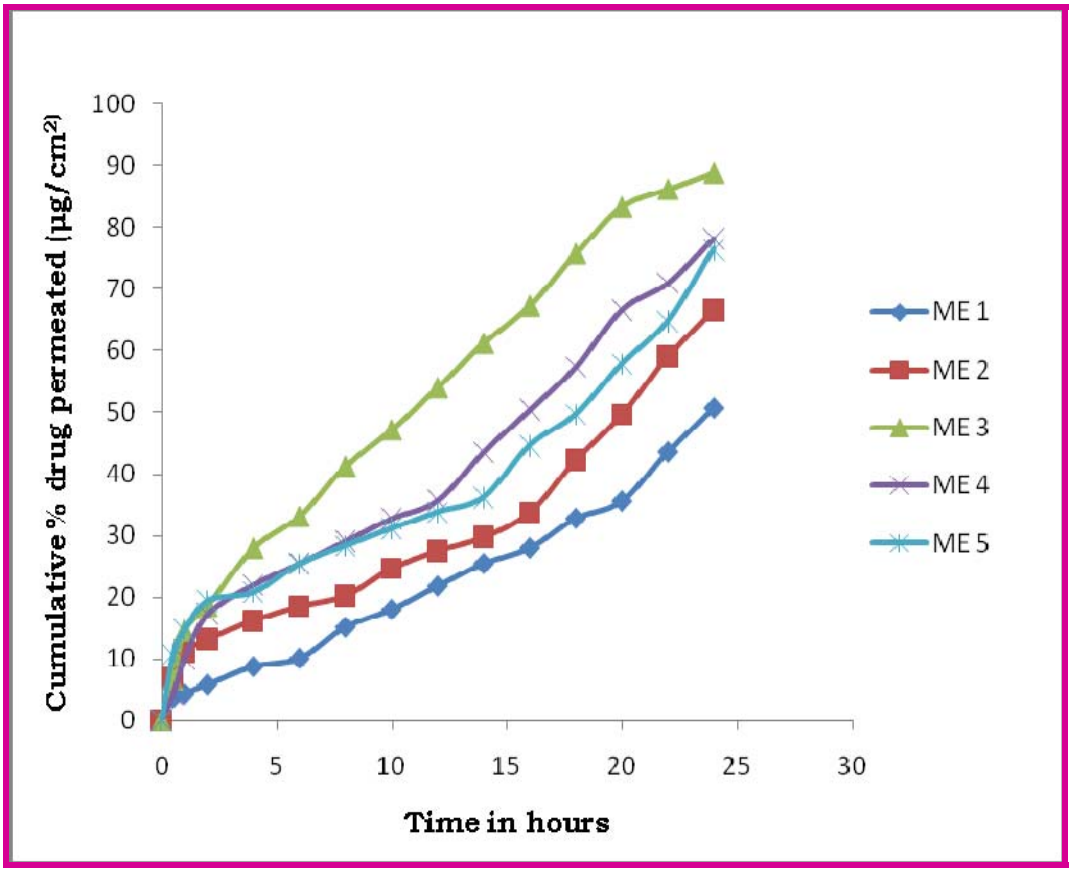
ME-3 Formulation possesses higher drug content compared to other ME formulations.

Table 36: Comparative *in vitro* Skin permeation rate of Lornoxicam Microemulsions

Time in hours	Cumulative % drug permeated (µg/cm^2)*				
	ME-1	**ME-2**	**ME-3**	**ME-4**	**ME-5**
0	0	0	0	0	0
0.5	3.71±0.12	7.06±0.032	6.59±0.42	4.22±0.12	10.70±1.02
1	4.32±0.02	11.03±0.42	14.56±0.31	10.02±0.02	14.70±0.34
2	5.84±2.11	13.15±2.12	18.59±2.10	17.23±0.31	19.39±0.15
4	8.76±0.21	16.22±1.25	27.96±0.24	21.98±1.02	20.79±0.52
6	10.09±1.13	18.52±0.02	33.12±0.15	25.44±1.32	25.41±0.12
8	15.14±0.02	20.25±1.01	41.24±1.02	29.05±2.01	28.36±0.32
10	18.02±0.21	24.65±0.21	47.14±0.21	32.72±0.02	31.14±2.53
12	21.80±1.04	27.53±1.31	53.98±2.25	35.68±0.12	33.80±1.28
14	25.44±0.01	29.73±0.20	61.19±0.20	43.53±0.21	36.22±0.28
16	27.96±0.02	33.77±0.35	67.24±1.01	50.34±1.20	44.61±0.2
18	32.76±2.24	42.23±2.25	75.68±0.13	57.30±2.03	49.66±1.26
20	35.57±0.31	49.62±0.14	83.24±0.20	66.56±0.41	57.80±0,02
22	43.53±0.24	59.03±0.21	86.12±2.02	70.92±0.25	64.76±1.25
24	50.63±0.02	66.56±2.32	88.79±0.15	78.13±1.02	76.40±0.15

***Values are mean** ±SD, n=3

Fig 38: Comparative *in vitro* Skin permeation rate of Lornoxicam Microemulsions



The comparative graph indicates ME-3 had higher skin permeation rate compared other microemulsion formulations.

Table 37: Model fitting of the in vitro Permeation data of various lornoxicam microemulsions

Formulations	r^2 value				
	Zero order	First order	Higuchi	Korsmeyer-peppas	Diffusional exponent (n)
ME-1	0.978	0.817	0.899	0.999	0.7
ME-2	0.941	0.817	0.845	0.563	1.9
ME-3	0.989	0.958	0.981	0.988	1.0
ME-4	0.982	0.817	0.933	0.644	2.0
ME-5	0.955	0.817	0.873	0.913	1.1

Release kinetics:

Table 38: Release kinetics of ME-1

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. % Drug Release	log %Cumu. of drug remaind
0.50	0.3010	0.71	3.711712	0.5696	1.9836
1.00	0.0000	1.00	4.324324	0.6359	1.9808
2.00	0.3010	1.41	5.837838	0.7663	1.9739
4.00	0.6021	2.00	8.756757	0.9423	1.9602
6.00	0.7782	2.45	10.09009	1.0039	1.9538
8.00	0.9031	2.83	15.13514	1.1800	1.9287
10.00	1.0000	3.16	18.01802	1.2557	1.9137
12.00	1.0792	3.46	21.8018	1.3385	1.8932
14.00	1.1461	3.74	25.44144	1.4055	1.8725
16.00	1.2041	4.00	27.96396	1.4466	1.8575
18.00	1.2553	4.24	32.75676	1.5153	1.8276
20.00	1.3010	4.47	35.56757	1.5511	1.8091
22.00	1.3424	4.69	43.53153	1.6388	1.7518
24.00	1.3802	4.90	50.63063	1.7044	1.6935

Fig 39: ME-1 Zero order plot

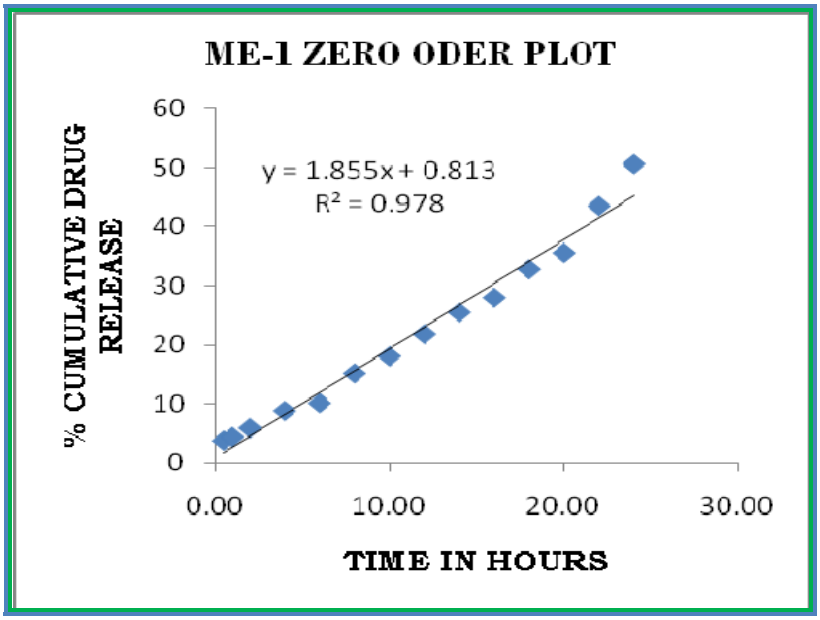


Fig 40: ME-1 First order plot

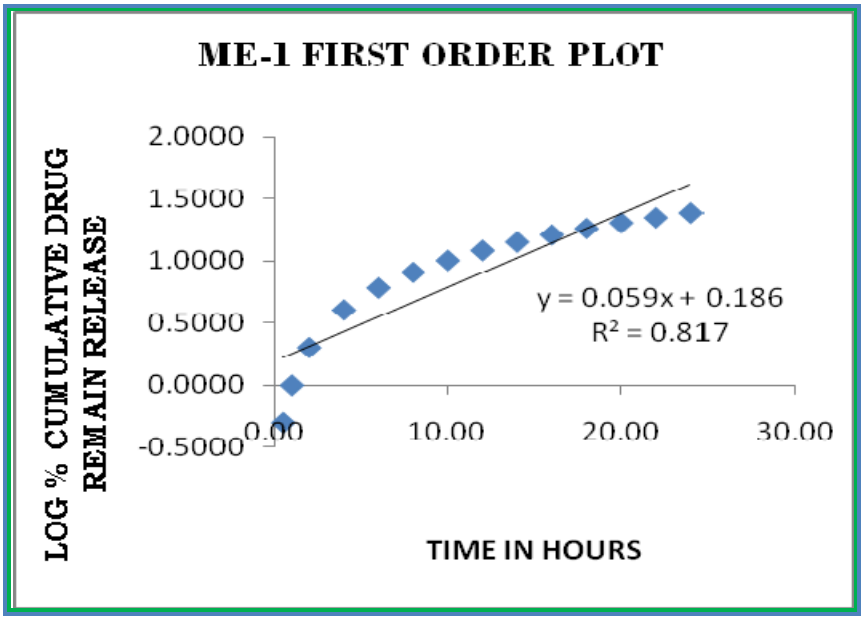


Fig 41: ME-1 Higuchi plot

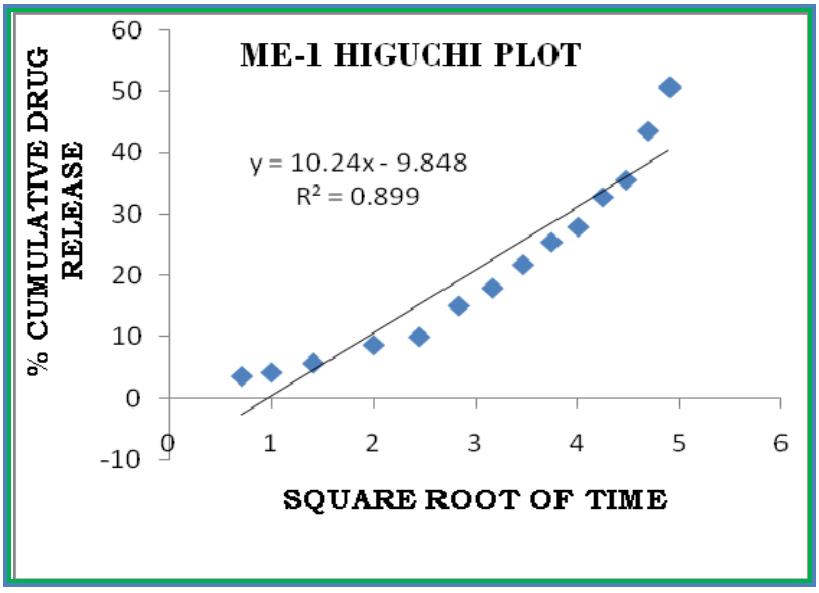


Fig42 : ME-1Korsemeuer-peppas model

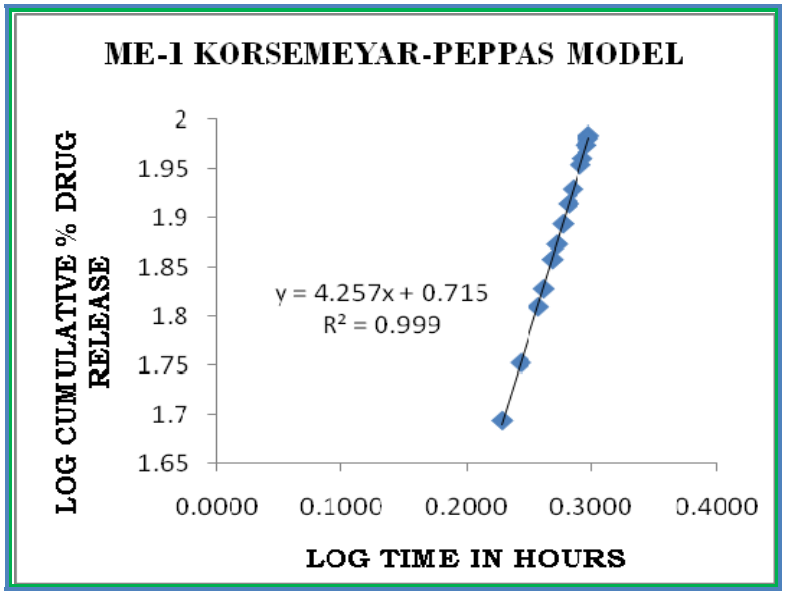


Table 39: Release kinetics of ME-2

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. % Drug Release	log %Cumu. of drug remaind
0.50	-0.3010	0.71	7.063063	0.8490	1.9682
1.00	0.0000	1.00	11.02703	1.0425	1.9493
2.00	0.3010	1.41	13.15315	1.1190	1.9388
4.00	0.6021	2.00	16.21622	1.2099	1.9232
6.00	0.7782	2.45	18.52252	1.2677	1.9110
8.00	0.9031	2.83	20.25225	1.3065	1.9017
10.00	1.0000	3.16	24.64865	1.3918	1.8771
12.00	1.0792	3.46	27.53153	1.4398	1.8601
14.00	1.1461	3.74	29.72973	1.4732	1.8468
16.00	1.2041	4.00	33.76577	1.5285	1.8211
18.00	1.2553	4.24	42.23423	1.6257	1.7617
20.00	1.3010	4.47	49.62162	1.6957	1.7022
22.00	1.3424	4.69	59.02703	1.7711	1.6125
24.00	1.3802	4.90	66.55856	1.8232	1.5243

Fig43 : ME-2 Zero order plot

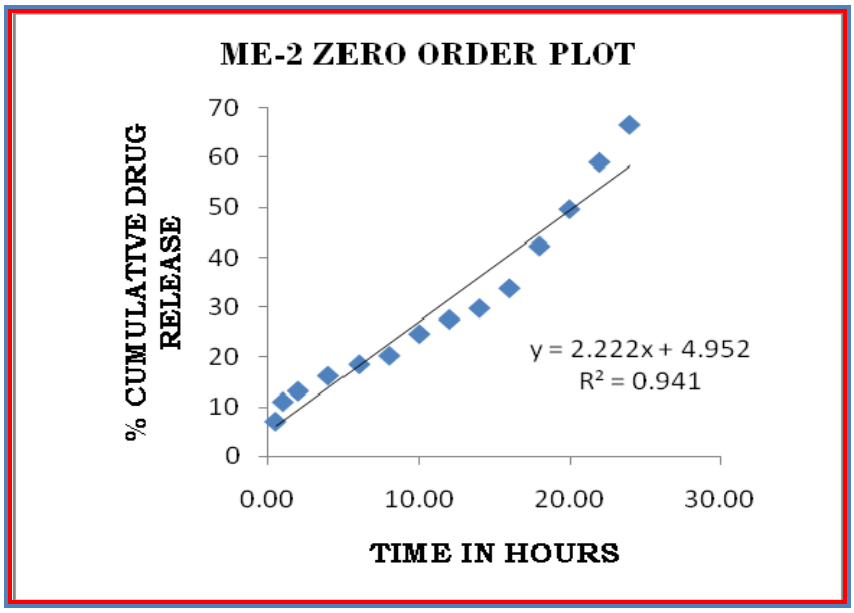


Fig 44: ME-2 First order plot

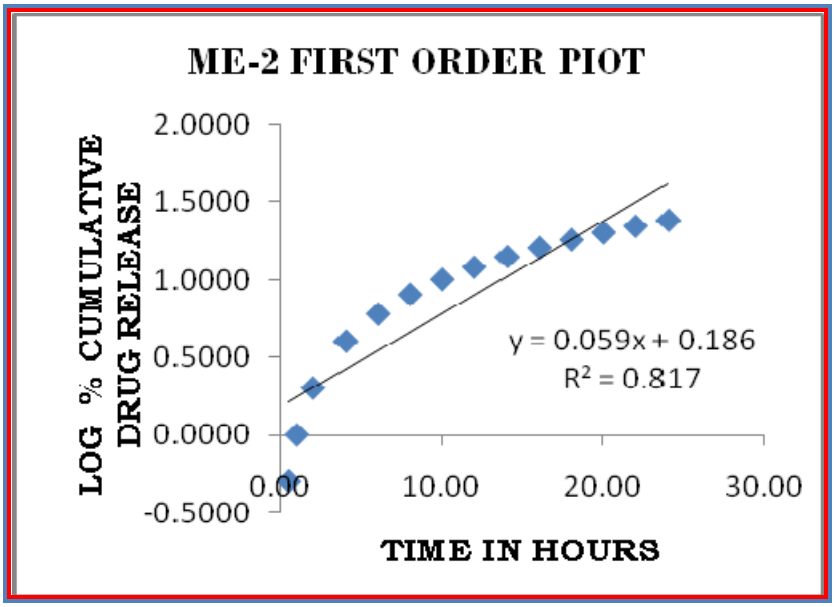


Fig 45: ME-2 Higuchi plot

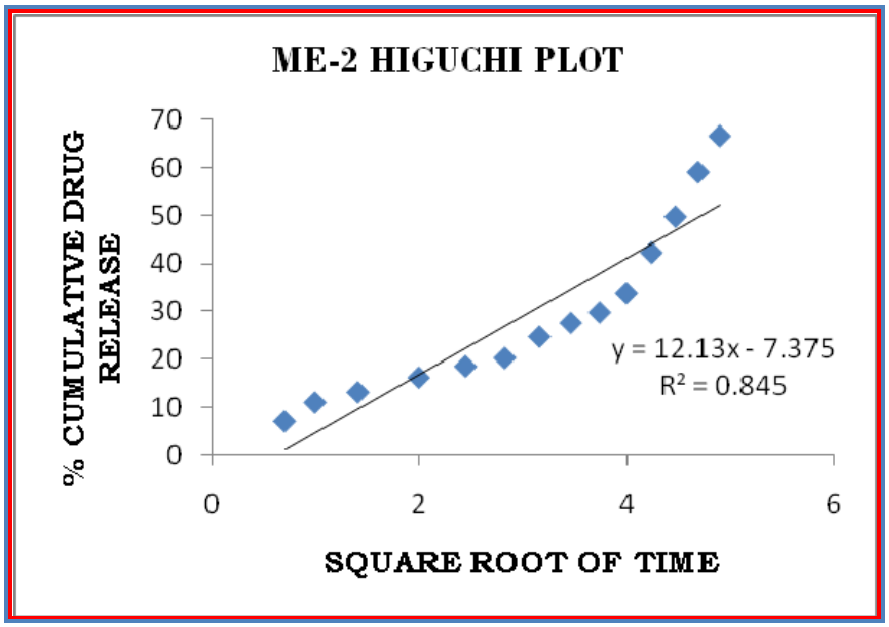


Fig 46: ME-2 Korsemeuer-peppas model

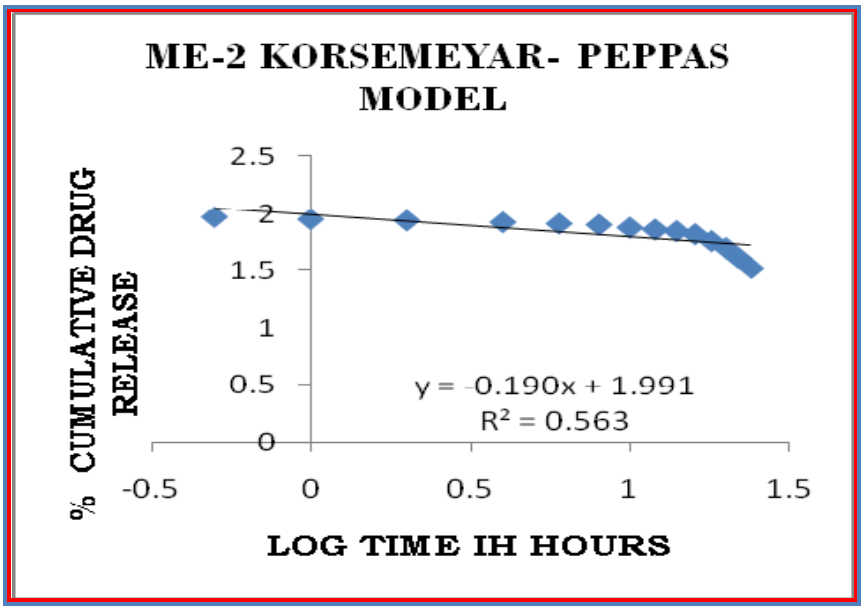


Table 40: Release kinetics of ME-3

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. % Drug Release	log %Cumu. of drug remaind
0.50	0.3010	0.71	6.59	0.8189	1.9704
1.00	0.0000	1.00	14.55	1.1629	1.9317
2.00	0.3010	1.41	18.59	1.2693	1.9107
4.00	0.6021	2.00	27.96	1.4465	1.8576
6.00	0.7782	2.45	33.11	1.5200	1.8254
8.00	0.9031	2.83	41.24	1.6153	1.7691
10.00	1.0000	3.16	47.13	1.6733	1.7232
12.00	1.0792	3.46	53.98	1.7322	1.6629
14.00	1.1461	3.74	61.18	1.7866	1.5891
16.00	1.2041	4.00	67.24	1.8276	1.5153
18.00	1.2553	4.24	75.67	1.8789	1.3861
20.00	1.3010	4.47	83.24	1.9203	1.2243
22.00	1.3424	4.69	86.12	1.9351	1.1424
24.00	1.3802	4.90	88.79	1.9484	1.0496

Fig 47: ME-3 Zero order plot

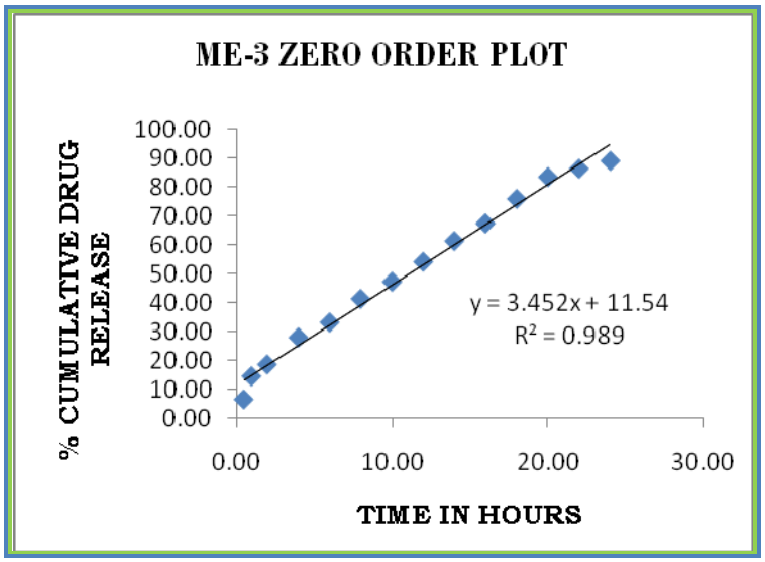


Fig 48: ME-3 First order plot

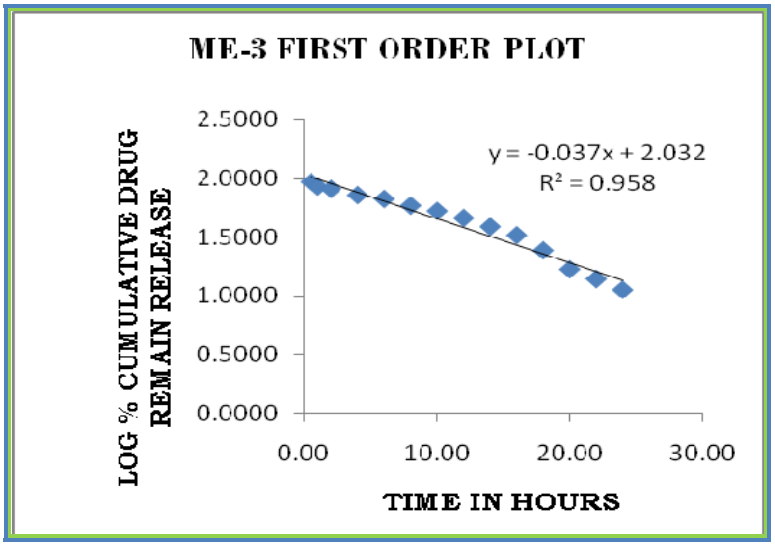


Fig 49: ME-3 Higuchi plot

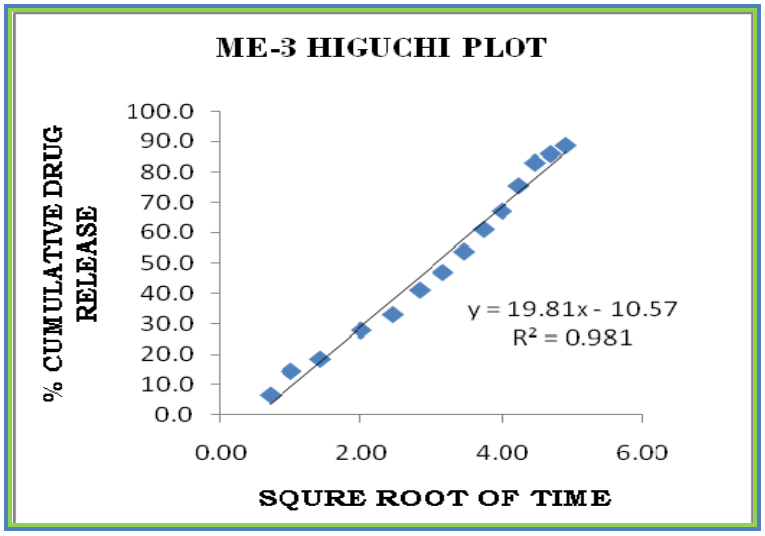


Fig 50: ME-3 Korsemeuer-peppas model

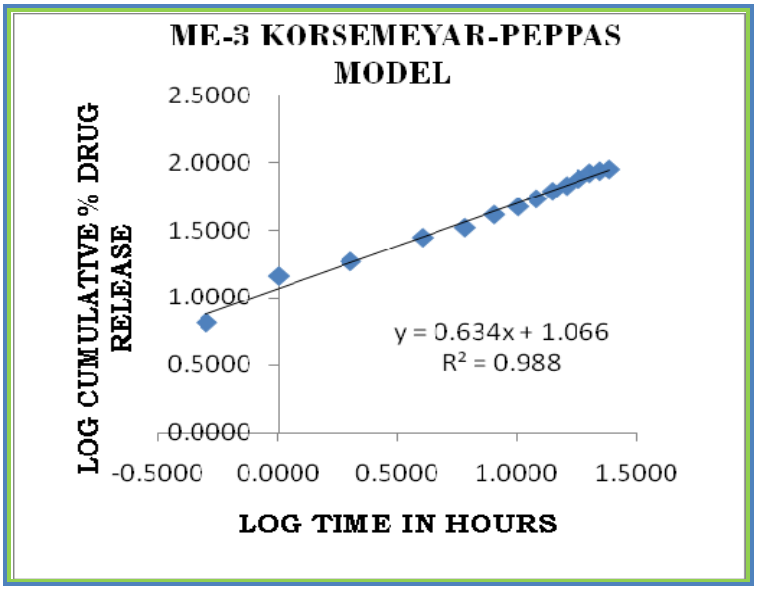


Table 41: Release kinetics of ME-4

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. % Drug Release	log %Cumu. of drug remaind
0.50	-0.3010	0.71	4.216216	0.6249	1.9813
1.00	0.0000	1.00	10.01802	1.0008	1.9542
2.00	0.3010	1.41	17.22523	1.2362	1.9179
4.00	0.6021	2.00	21.98198	1.3421	1.8922
6.00	0.7782	2.45	25.44144	1.4055	1.8725
8.00	0.9031	2.83	29.04505	1.4631	1.8510
10.00	1.0000	3.16	32.72072	1.5148	1.8279
12.00	1.0792	3.46	35.67568	1.5524	1.8084
14.00	1.1461	3.74	43.53153	1.6388	1.7518
16.00	1.2041	4.00	50.34234	1.7019	1.6960
18.00	1.2553	4.24	57.2973	1.7581	1.6305
20.00	1.3010	4.47	66.55856	1.8232	1.5243
22.00	1.3424	4.69	70.91892	1.8508	1.4636
24.00	1.3802	4.90	78.12613	1.8928	1.3399

Fig 51: ME-4 Zero order plot

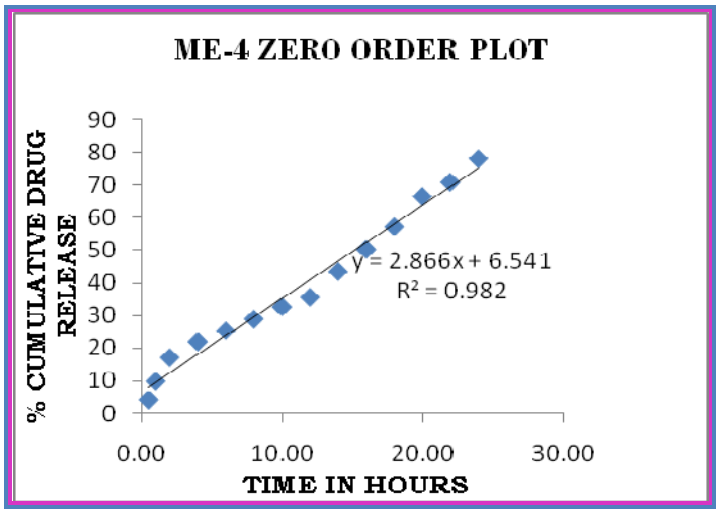


Fig52 : ME-4 First order plot

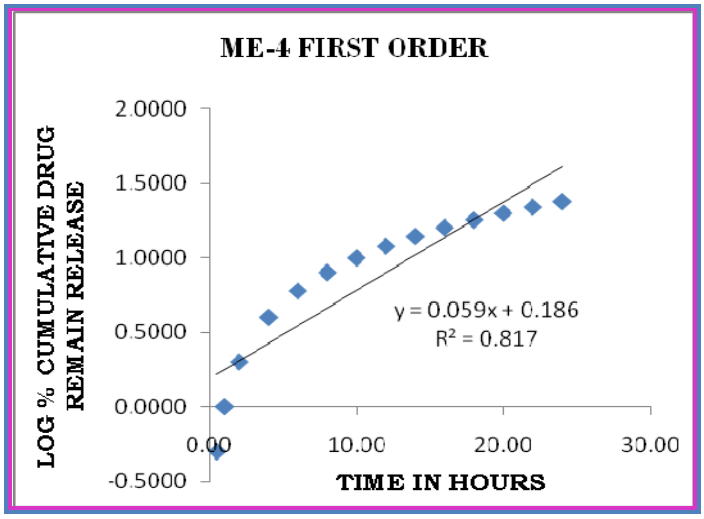


Fig 53: ME-4 Higuchi plot

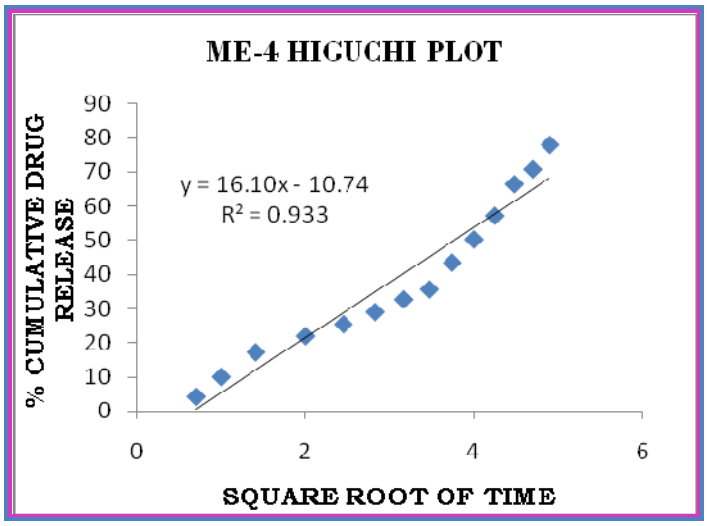


Fig 55: ME-4 Korsemeuer-peppas model

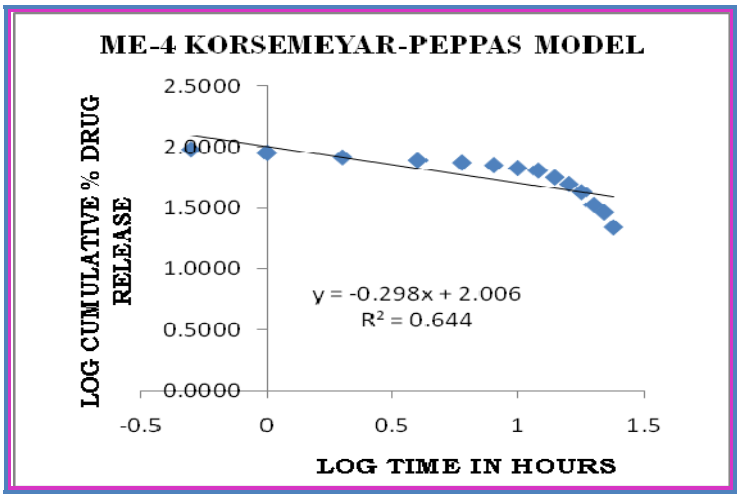


Table 42: Release kinetics of ME-5

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. % Drug Release	log %Cumu. of drug remaind
0.50	-0.3010	0.71	10.7027	1.0295	1.9508
1.00	0.0000	1.00	14.7027	1.1674	1.9309
2.00	0.3010	1.41	19.38739	1.2875	1.9064
4.00	0.6021	2.00	20.79279	1.3179	1.8988
6.00	0.7782	2.45	25.40541	1.4049	1.8727
8.00	0.9031	2.83	28.36036	1.4527	1.8552
10.00	1.0000	3.16	31.13514	1.4933	1.8380
12.00	1.0792	3.46	33.8018	1.5289	1.8208
14.00	1.1461	3.74	36.21622	1.5589	1.8047
16.00	1.2041	4.00	44.61261	1.6495	1.7434
18.00	1.2553	4.24	49.65766	1.6960	1.7019
20.00	1.3010	4.47	57.8018	1.7619	1.6253
22.00	1.3424	4.69	64.75676	1.8113	1.5471
24.00	1.3802	4.90	76.3964	1.8831	1.3730

Fig 55: ME-5 Zero order plot

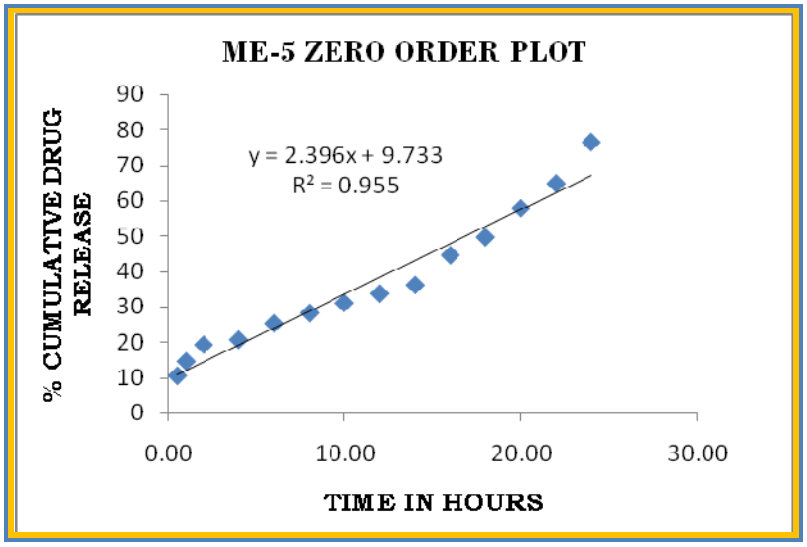


Fig 56: ME-5 First order plot

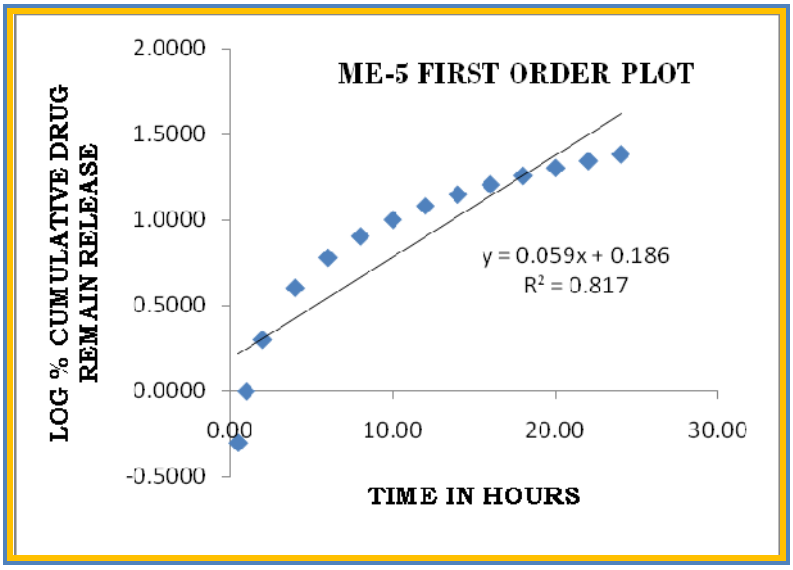


Fig 57: ME-5 Higuchi plot

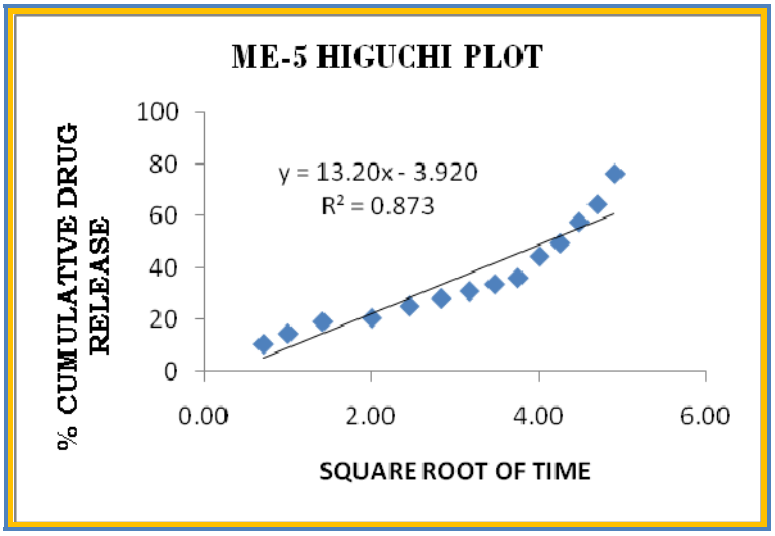
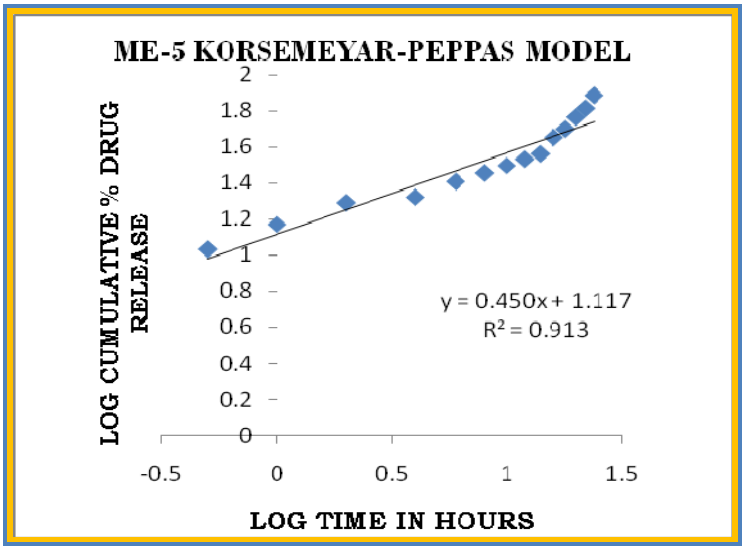


Fig 58: ME-5 Korsemeuer-peppas model



In vivo Study

Anti-inflammatory activity of ME-3

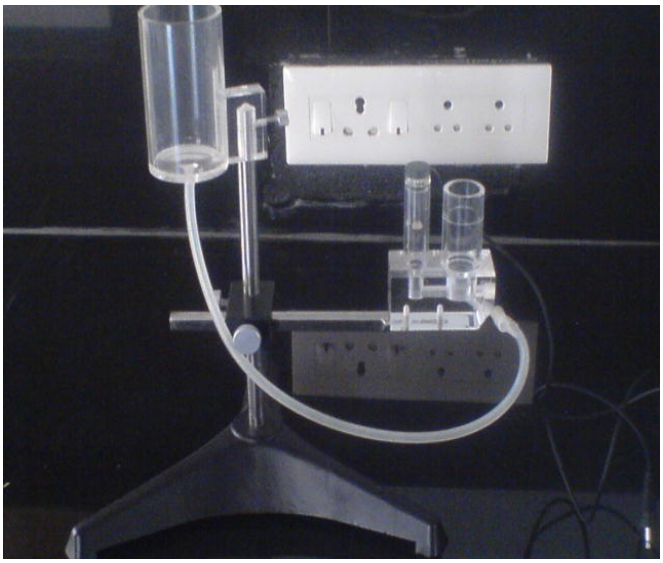


Fig 59: Digital plethysmometer

Fig 60: Testing of Wistar rat

Fig 61: Before treatment of ME-3 Formulation

Fig 62: After treatment of ME-3 Formulation

Table 43: Anti inflammatory activity of Lornoxicam against Carragenin induced Paw Edema in Wistar Rats

Treatment	% increase in paw volume mean ± S.D (n=3)				% inhibition in paw volume
	Post insult time of assay in minutes				
	0	60	120	180	
Control (0.5 ml/kg)	30.91 ± 1.53	69.32 ± 3.12	97.83 ± 8.13	108.59 ± 9.09	--
Lornoxicam Microemulsion ME-3 (10 mg/Kg)	29.74 ± 1.58	46.64*** ± 3.3	75.8*** ± 5.3	64.42*** ± 3.91	40.67
Standard-Indomethacin (10 mg/kg)	28.46 ± 0.92	33.8 ± 1.83	38.8 ± 2.32	43.2 ± 3.21	60.21

Data presented are Mean ±SD

p-value *<0.01; **<0.001; *<0.0001 Vs Control group using paired 't" test**

Fig 63: % of Increase in Paw volume

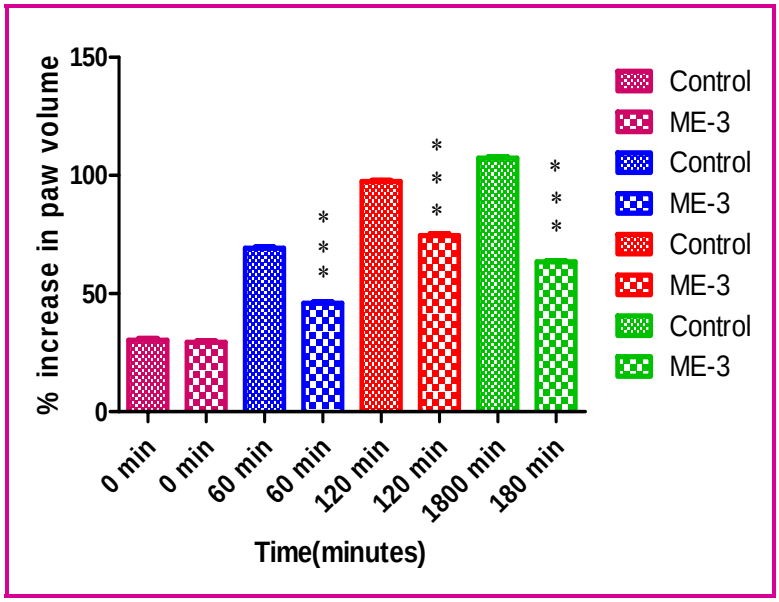


Fig 63: presented are Mean ±SD

P-value *<0.01; **<0.001; *<0.0001 Vs Control group using paired 't" test**

Stability Study:

S.No	Formulation ME-3	Before storage	Stored at 40°C ± 2°C and 75%±5% RH		
			1st month	2nd month	3rd month
1	Drug content (%)	98.54	98.23	98.01	97.36
2	pH	6.42	6.31	6.01	5.65
3	Viscosity(cps)	91.4	90.2	90	90

Fig 44: Stability study

Discussion:

Transdermal drug delivery systems facilitate the passage of therapeutic quantities of drug substances through the skin and into the general circulation for their systemic effects. Topical administration of drugs with systemic effect can have advantages over other methods for several reasons, one of which is the avoidance of hepatic first-pass metabolism of the drug and related toxicity effects, controlling the rate of delivery and modulating distribution of drug in the systemic circulation. In developing a transdermal delivery system, two criteria are considered: one is achieving adequate flux across the skin and the other is minimizing the lag time in skin permeation.

Transdermal delivery of microemulsion system, which composed of non-irritating, pharmaceutically acceptable ingredients. Microemulsion was prepared by water titration method using oleic acid as oil phase, tween-20 as surfactant and propylene glycol as co-surfactant

Different oils, surfactants and co-surfactants were screened to select ideal components of microemulsions with good solubility and excellent skin penetration of lornoxicam. The solubility of lornoxicam was highest in oleic acid followed by olive oil, castor oil, and isopropyl myristate, isopropyl palmitate.

The use of **Oleic Acid** is advantageous because it increase skin permeability by two mechanistic scenarios of the enhancer; (a) lipid fluidization, and (b) lipid phase separation, oleic acid is a model skin permeation enhancer, Oleic acid facilitates penetration into the skin by disrupting the fluidity of the stratum corneum. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin. The results of solubility study have shown table 15.

Non–ionic surfactant was selected because they are generally less toxic, produce less skin irritation. The HLB value of the O/W type of microemulsion (9-12).HLB value of Tween-20(16.7), the required HLB for O/W type of emulsion for oleic acid (17) and span-20 HLB value (8.6).The hydrophilic non- ionic surfactant, **Tween-20** was chosen to formulate these microemulsion systems to provide a better permeation profile. The different co-surfactants like butanol, ethanol and isopropyle alcohol are used in place of propylene glycol but clarity and cumulative percent release was found to be expected with **propylene glycol.**

In microemulsion, the co-surfactant lowers the interfacial tension of the surfactant film, resulting in a more flexibility and dynamic layer system. The thermodynamic driving force for the release reflects shows the relative activities of the drug in different phase. Since drug can be release from the internal phase to external phase and then from external phase to the skin, the relative activities may monitor the skin permeation flux.

In addition, the surfactant and co-surfactant may exist in each phase, so lornoxicam can partly solubilized in external phase. The depletion of lornoxicam may be from the external phase because of the permeation in to the skin can be supplemented by the release of lornoxicam from the internal phase. However, the oily mixtures of oleic acid, tween-20 and propylene glycol led to increased in drug solubility. After extensive screening for physical characteristics and appearance, final ratios of surfactants- cosurfactants were decided. The data of selection of surfactants and cosurfactants is given in Table 16.

In order to identify the optimum ME formulation containing oleic acid, Tween -20, propylene glycol were selected from the trial formulations ratios (1:1, 2:1) in table17and18. (F1-F10) trial formulations in two ratios (1:1, 2:1) of microemulsion formulations were prepared by varying the amount of surfactant/co-surfactant.

In 1:1 and 2:1 ratios of trial formulations stable microemulsions were not formed. Turbid and conventional emulsions based visual observation. From ten trial formulations one **(F8) formulation** were selected on 2:1 ratio for optimizing the formulations. Finally drug, surfactant and co-surfactant is kept constant and oil amount was changed. The compositions of microemulsions are given in table 19.

The microemulsion (ME-1 to ME-5 were subjected to the study of **Opticle transparency**.ME-3formulation were optically clear, transparent and elegant in appearance when compared to the other microemulsion formulations Fig 27.

The **pH** values of ME-1 to ME-5 (6.12±0.04, 5.75±0.03, 6.42±0.02, 5.81±0.03, 4.35±0.06) units only. In all cases pH showed the smallest changes. The pH value of optimized lornoxicam microemulsion formulation (ME-3) was 6.42±0.02 (table 32) and is suitable for topical as well as transdermal application because the pH of skin in the range 5.5 to 7.0.

It was clear from the **viscosity** of microemulsion systems (ME-1 to ME-5) that the developed formulations have gradually increased (52.6±0.6cps, 75.3±0.8cps, 91.4±0.4cps, 103.5±0.5cps, 118.2±0.2cps). All samples exhibited Newtonian flow behaviour, as expected from microemulsions. It could be noted that the viscosity values tended to increase slightly when the water concentrations increased or when the system turned into oil/water type because oil/water microemulsions have higher viscosities than those of water/oil systems in table 33.

The **Centrifugation stress test** of formulations ME-3 shows good physical stability and no phase separation when were centrifuged at 2000 rpm for 60mins Fig 32.

The morphology of MEs was characterized using **Transmission Electron Microscopy** (Fig.33).The TEM image of optimized best formulation showed that globules were spherical in shape and had smooth surface. The results TEM further indicated the excistance of an isotropic dispersion of spherical droplets, leading to the assumption of inverse micelles because of the proporsion of the constituents.

The particle size distribution of the best microemulsion formulation (ME-3) ranged from 0.276μm to 1.231 μm with the average droplet size of (ME-3) 0.78μm. The result shows that the droplet diameter decreases with increasing ratio of oil and surfactant: co-surfactant. Due to the small droplet size of (ME-3) microemulsion, its surface areas are assumed to be high. Therefore, droplets of microemulsion settled down to close contact with the skin providing high concentration gradient and improved ornoxicam permeation from formulation (ME-3).

The average droplet size of the best microemulsion formulation (ME-3) was determined by **Atomic Force Microscopy**. The average droplet sizes of ME-3 0.88μm and 0.45μm. The droplet size distribution curves of ME-3 were presented in Fig: (a.34), (b.35).

The mean percent **drug content** in microemulsion formulations (ME-1 to ME-5) was found to be respectively. (82.42± 0.32%, 93.12± 0.54%, 98.54± 0.26%, 90.21± 0.46%, 86.34 ±0.28%). ME-3 was exhibited 98.54± 0.26% higher drug content then other formulations Table 35.

The permeation capability of the microemulsion formulations were evaluated by conducting the ***in-vitro* skin permeation** experiments. ME (1-5) lornoxicam microemulsions were studied for *in vitro* skin permeation through excised goat skin. The amount of lornoxicam permeated through excised goat skin over 24-hour period was plotted against the function of time (Fig 38), the permeation fluxes (μg/cm^2/hour) for all these microemulsions through the goat skin were determined. The determined permeation fluxes are given in Table36. Among all formulations, the highest permeation flux of μg/cm2/hour was observed in case of formulation ME-3.

The *in vitro* lornoxicam permeation data from microemulsions containing lornoxicam through excised goat skin were evaluated kinetically by various mathematical models like zero-order, first-order, Higuchi, and Korsmeyer-Peppas model .The results of the curve fitting into these above-mentioned **Mathematical models** indicate the in vitro Lornoxiam permeation behavior of Lornoxicam microemulsions (ME-1 to ME-5) (Table 37).

When respective correlation coefficients were compared, ME-1, ME-2, ME-3, ME-4 and ME-5. ME-3 followed the zero-order release (r2 = 0.982) over a period of 24 hours. Again, the Korsmeyer-Peppas model was employed in the in vitro lornoxicam permeation behavior analysis of these formulations to find out permeation mechanisms: Fickian (nonsteady) diffusional release when $n \leq 0.5$, case-II transport (zero-order) when $n \geq 1$, and non-Fickian, “anomalous” release when the value of n is in between 0.5 and 1.

The determined values of diffusion exponent (n=0.1) in (Table37). ME-3 indicating that the drug permeation from lornoxicam microemulsion followed the non-Fickian, “anomalous” mechanism.

Stability of the prepared microemulsion formulations was assessed using accelearated temperature study. The drug content, pH, viscosity of the best formulation ME-3 were subjected to stability studies at 40°C/75% RH up to 3 months. the results are summarized in table 44. ME-3 Sample showed excellent results in these studies. Drug degradation was found to be in the range (98.23%, 98.01%, and 97.36%) after three months. Viscosity values after three months compared to the initial viscosity were in the range 0-1cps, while pH changed 6-5 units only. In all cases, ME-3 showed the smallest changes in these parameters. Overall results from the stability studies indicated that the microemulsions were chemically stable for three months.

Transdermal treatment of the rats with lornoxicam significantly inhibits the oedema size induced by carrageen injection into the sub-plantar area of the right hind paw for each rat. It is observed from figures (61) and (62) that the groups that treated with lornoxicam microemulsion formulation exhibit a maximum percent oedema inhibition after 1hr, 2hr, 3hr, respectively, which is lower than that of control group (Fig 63).

CONCLUSION

The study demonstrates that the microemulsion formulation can be employed to improve the solubility of the poorly water soluble drug. Oleic acid has consumed maximum amount of lornoxicam and thus chosen as a vehicle for microemulsion oil phase. Tween-20 and propylene glycol at appropriate ratios were selected as an ideal surfactant and co-surfactant.

Lornoxicam Microemulsion was formulated as controlled release dosage form for the period of 24hrs and also reduced the side effects produced by oral conventional doses.

Characterization of the selected ME-3 formulation containing oleic acid (6%), Tween-20/propylene glycol (30%), showed their stability after exposure to a centrifuge stress test. Their viscosity and droplet particle size showed their suitability for transdermal application and their pH values are within the physiological range.

Drug content of the formulation revealed the % of the formulation98.54±0.26%.

This study highlighted the efficacy of lornoxicam ME-3 for enhanced in vitro transdermal permeation through goat skin. The skin permeation of the ME-3 formulation follows zero order kinetics and fit for the korsemeyer-peppas model and follows non-fickian (anomalous).

From the obtained results in this study, we can conclude that: Group: 1 (animal groups treated with lornoxicam microemulsion) and Group 2: (animal groups treated with control drug).lornoxicam microemulsion (ME-3) produced maximum % oedema inhibition after 1hr, 2hr, 3hr respectively, and then continued significantly for 3hrs than these control group.

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